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### ADDENDUM

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# KAGZI LIME: AN INDICATOR PLANT OF THE CITRUS 'DECLINE' VIRUS IN INDIA

S. P. Capoor,

(Accepted for publication July 10, 1961)

The 'decline' of Mosambi orange in the Bombay State was observed to be caused by the tristeza virus first in the year 1958 (Vasudeva and Capoor, 1958), although investigations on the causes of 'decline' have been carried out extensively at this Institute since January 1955. In the beginning the method that was followed for testing the presence of the virus in declining trees was to bud or graft a prepared combination of Sweet orange on Sour orange rootstock with the bud-wood to be tested. For this combination seedlings of Sweet and Sour oranges were raised from seed under insect-free conditions, and, as far as plausible, only nucellar seedlings were grafted and grown in pots according to the method described by Stubbs (1952). Later, screening of the suspected citrus trees was carried out on seedlings of the West Indian Key lime. Following the discovery of the lime disease in the Gold Coast (Hughes and Lister, 1949), and the reported identity of the lime disease and the tristeza induced stem-pitting of grapefruit in South Africa (McClean, 1950) and the Quick Decline virus in California (Wallace and Drake, 1951), and also successful transmission of the tristeza virus to the Key lime seedlings in Brazil through *Aphis citricidus* (Kirk.) (Costa, Grant, and Moreira, 1950), the Mexican or the West Indian Key lime (*Citrus aurantifolia* (Christman) Swingle) has been used as an indicator plant for the tristeza virus. Symptoms of the disease in Key lime seedlings appear within 30 to 40 days and are characteristic of the tristeza virus alone. These consist of (1) chlorotic spots and dashes along the lateral veins and veinlets, best seen in immature leaves viewed against transmitted light, (2) pits and grooves in the decorticated woody cylinder, and (3) stunting of the plant as a whole.

Great difficulty was experienced, however, on account of shortage of Key lime seedlings that were required for indexing a large number of budsticks obtained from suspected trees of citrus varieties growing in various localities in the Bombay State and the adjoining areas. This was mainly due to the fact that seed of Key lime had to be obtained from abroad and was in short supply. Consequently, seedlings of about the same age could not be made available regularly for indexing in order to have uniform results. Screening tests were, therefore, carried out at Poona using nucellar seedlings of citrus species commonly available in India for their susceptibility to the 'decline' virus in order to discover species that might serve as useful indicator or differential hosts. Seedlings were grown from seed and reared in pots under insect-free conditions. Eight to 12 uniformly growing seedlings of each species were infected by budding, or grafting, or by both methods with budwood obtained from a known source of the 'decline' virus. Budded or grafted plants were kept for a week to 10 days on the glasshouse bench inside a moist drum covered

with a glass sheet on top. These plants were examined periodically for specific symptoms which might be of some diagnostic value.

Of the 12 citrus species, tested only two, i.e. Kagzi or Acid lime (*Citrus aurantifolia* (Christman) Swingle) and Eureka lemon (*Citrus limon* (Linn.) Burm.) produced diagnostic symptoms, 5 were infected, and the rest did not show symptoms (Table 1). These will be indexed by back grafting to seedlings of West Indian and Kagzi limes in order to ascertain if they are tolerant and carying the virus without producing any symptoms, or immune.

Table I. Reaction of *Citrus* species and varieties to infection by the 'decline' virus when inoculated by budding or grafting.

| Species | Symptoms produced |
|---|---|
| *Citrus aurantifolia* (Chistm.) Swing.; Kagzi lime. | Chlorotic dashes on veins and veinlets, stunting, and stem-pitting. |
| **Citrus limon* (Linn.) Burman; Eureka lemon. | Chlorosis and chlorotic rings, necrosis of veins, severe stunting and shortening of internodes. |
| **C. limon;* Rough lemon | No leaf symptoms, but some retardation of growth. |
| **C. limon;* Standard Sour lemon | Severe stunting, chlorosis of leaves, slow wilting and death of plants. |
| **C. limon;* Sweet lime. | No apparent symptoms. |
| *Citrus paradisi* Macf.; grapefruit. | Yellowing and cupping of leaves, corking and splitting of veins, severe stunting and stem-pitting. |
| **Citrus aurantium* L.; Sour orange. | Severe stunting, downward rolling of leaves, and corking and spliting of veins. |
| **Citrus sinensis* Osbeck; Mosambi. | Mild corking and splitting of veins. |
| * *Citrus reticulata* Blanco; Mandarin orange. | Some retardation of growth. |
| **Citrus pennivisciculata* Tanaka | No apparent symptoms. |
| **Citrus mitis;* Calamondin | General chlorosis, severe stunting, and gradual decline leading to death. |
| ***Citrus aurantifolia* (Christm). Swing.; West Indian or Mexican Key lime. | Chlorotic dots and dashes on veins and veinlets, mild chlorosis of leaves, stunting and severe stem-pitting. |

* These have not yet been examined for the stem-pitting symptoms.

** Inoculated for confirmation.

Symptoms of the disease in Kagzi lime seedlings appear within 30 to 60 days of budding or inoculation through the aphid vector (Vasudeva *et al.*, 1959), and develop in the following sequence: 1) chlorotic dashes of the veins and veinlets of developing young leaves seen clearly against transmitted light, (2) formation of brownish streaks on the wood of twigs gradually developing into pits, and (3) marked dwarfing of seedlings. The infected seedlings also show general chlorosis and weakened growth. Though symptoms of disease induced by the 'decline' virus in Kagzi lime are identical with those produced by the tristeza virus in the Key lime seedlings the Kagzi lime is better suited to our conditions than the West Indian Key lime in some respects. The chlorotic dashes and dots on veins and veinlets of the infected Kagzi lime seedlings are very prominent and sparsely distributed over the leaf surface, and, secondly, they last longer than those formed on leaves of the Key lime plants and appear to stand higher temperature which usually prevails in our glasshouses. In addition, seed of Kagzi lime is available locally and throughout the year.

Symptoms of vein clearing induced by the tristeza virus have been reported in leaves of *Aeglopsis chevalieri* Swing., *Afraegle paniculata* (Schum.) Engl., *Citrus combava* Raf., *Citrus hystrix* DC., and *Pamburus missiones* (Wt.) Swing. including Key lime in Argentina (Knorr, 1956), and in the Egyptian sour lime in Israel (Reichert and Bental, 1957), but the evidence of the presence of tristeza virus in areas where it was not known to be present before has been recorded only on the Key lime indicator plants (Grant and Schneider, 1953; Wallace *et al.*, 1956; Nour-Eldin and Bishay, 1958). In this Laboratory also the confirmation of the presence of the tristeza virus responsible for the 'decline' of citrus in India was done on Key lime plants (Vasudeva and Capoor, 1958; Vasudeva *et al.*, 1959). Since the symptoms induced by the 'decline' virus in Kagzi lime seedlings are identical with those produced in Key lime seedlins by the tristeza virus, Kagzi lime has proved to be an equally efficient indicator plant for the tristeza virus and is being used extensively at this Laboratory in routine screening as well as transmission tests of the 'decline' virus. Incidentally, Kagzi lime has been experimentally demonstrated to be susceptible to the tristeza virus for the first time.

Acknowledgement. The author wishes to express his gratitude to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology for his guidance and keen interest during the progress of this study and for critically reading the manuscript. He is also indebted to Dr. L. C. Knorr of the Citrus Experiment Station, Lake Alfred, Florida, U.S.A., for the supply of Keylime seed.

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# RESISTANCE TO MOSAIC IN CERTAIN CHILLI VARIETIES

G. P. S. Anand, M. D. Mishra and Amar Singh

(Accepted for publication July 10, 1961)

Mosaic is one of the most widely spread and serious virus diseases of the chilli crop in India. The disease is characterised by dark green and chlorotic patches scattered all over the leaf-lamina, with slight puckering and blistering. Occasionally there is considerable reduction in the leaf size, so much so that the leaves are reduced almost to a filiform shape. The affected plants bear fewer number of flowers and fruits. The fruits on the diseased plants are distorted and rough in texture. The incidence of the disease in the cultivators' fields in the year 1957-58 was about 20 – 30 per cent. However, in the field-plots of Botany Division of this Institute the incidence was found to be as high as 72 per cent in the year 1959-60.

The causal virus was studied by Jha and Raychaudhuri (1956), who reported *Aphis gossypii* Glov. to be its vector. Subsequently Nariani and Sastry (1958) reported *Aphis evonymi* Fabr. and *Myzus persicae* Sulz. as two additional vectors of the virus.

Due to the serious nature of the disease an elaborate programme of testing a larger number of chilli varieties and *Capsicum* spp. against the causal virus was drawn out during 1956-60, in an attempt to search for possible sources of resistance. Regular survey of a large collection of about 400 different varieties and strains of chillies grown in the field plots of Botany Division at the Indian Agricultural Research Institute and in the cultivators' fields in the villages around Delhi, Sonepat and Panipat was undertaken in the first instance. The varieties which showed freedom from the disease under field conditions were tested for their resistance in the following years under controlled glasshouse conditions.

Seedlings, raised from the seed under insect-proof conditions, were mechanically inoculated with the chilli mosaic virus by juice-inoculation. The varieties, which did not show apparent symptoms on artificial inoculation were indexed on susceptible chilli variety NP46A to determine if they were symptom-less carriers. In all, 132 varieties of chillies and six species of *Capsicum* were tested, of which the following proved to be susceptible;

NP 3 red, NP 3 orange, NP 5, NP 6, NP 9, NP 10, NP 11, NP 19, NP 20, NP 21, NP 22, NP 23, NP 24, NP 26, NP 28, NP 29, NP 32, NP 34, NP 35, NP 39, NP 40, NP 41, NP 45, NP 46A, NP 47. NP 49, NP 50, NP 51, NP 52, NP 55, NP 56, NP 60, NP 62, NP 66, NP Hybrid 5–1–5, NP Hybrid 17–1–1,

IC 576, IC 1404, IC 2474, IC 2673, IC 3385, IC 3399, IC 3401, IC 3414, IC 3415, IC 3416, IC 3422, IC 3468, IC 3470, IC 3473, IC 3479, IC 3486,

IC 3522, IC 3523, IC 3526, IC 3593, IC 3690, IC 3772, IC 3972, IC 3975, IC 3978, IC 3980, IC 3981, IC 3986, IC 3989, IC 3991, IC 4574, IC 5544, IC 5698, IC 5700, IC 5701, IC 5901, IC 6531, IC 6554,

EC 8290, EC 8292, EC 8297, EC 8299, EC 8302, EC 8305, EC 8306, EC 3232, EC 12,520 (R 449),

45/1, 76, 156, 182/2, 197, 324/1, 327, 335, 5046–1, 5402–1, 5406–1, 5413–1, RS 1, RS 6, RS 8.

Bapatla, Kammireddipalam, Cuttack Red Erect, Cuttack Red Pendent, Bombay 76–1, Bombay 80–1, Bombay 469, Patna, Pusa, Ellichpur, Kangar Red, Kangra Orange, Hyderabad II, Narela 9 sp., Narela 9 (Selection 1), Kundli 1 (Selection 1), Kundli 1 (Selection 2), Kundli 3, Kundli 7 (Selection 1), Singola 7 (Selection 1), Suryamukh Red Erect.

Tobasco, Nigerian, Fresno, Floral gem, Santaka, Red Pearl, Red Chilli Erect and King of North.

*Capsicum annuum* L., *C. frutescens* L., *C. pendulum* Willd., *C. microcarpum* Cav., *C. sinensis* Jacq. and *C. pubscens* R. & P.

Five chilli varieties *viz.* Puri Red, Puri Orange, Kondiverum, $G_2$ and a local variety, proved to be resistant. The results were further confirmed by feeding viruliferous aphids (*Aphis gossypii* Glov.) on them.

ACKNOWLEDGEMENTS; Grateful thanks are due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology for his valuable suggestions, keen interest and encouragement. Thanks are also due to Shri T. K. Naraini for going through the manuscript and also to the Indian Council of Agricultural Research for financing the Scheme.

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# SOME CERCOSPORA SPECIES FROM INDIA-V *

G. Lall, H. S. Gill and R. L. Munjal

(Accepted for publication July 15, 1961)

In this article 12 species of Cercospora have been described 3 of which are new species, six are new records from India and three have new hosts. A number of species have economic hosts such as *Syzygium cuminii*, *Gossypium hirsutum*, *Helianthus annuus*, and *Averrhoa carambola*.

The specimens have been deposited in the Herb. Crypt. Ind. Orient., I.A.R.I., New Delhi and their respective numbers are indicated in the text.

*Cercospora avicularis* Wint., Jour. Mycol. 1 : 125, 1885.

On living leaves of *Polygonum aviculare* L. (Polygonaceae), Katrain, Kangra (Punjab), 15–11–1959, H. S. Gill and Gian Singh, H.C.I.O., No. 27051.

The fungus forms spots which turn reddish brown finally on both surfaces of the leaf. The conidiophores arise in fascicles from small stromata, are subhyaline to pale olivaceous brown, sparingly geniculate, and measure 3.5 – 4 x 25 – 58μ. The conidia are pale olivaceous, obclavate and measure 3.5 x 50 – 162μ.

*Cercospora beticola* Sacc., Nuov. Giron. Bot. Ital., 8 : 189, 1876; Sacc. 4 : 456, 1886; Syd. and McRae, Ann. Crypt. Exot. 2 : 263, 1930.

On living leaves of *Beta cicla* L. (Chenopodiaceae), Kulu, Kangra (Punjab), 15–11–1959, H. S. Gill and Gian Singh, H.C.I.O. No. 27049.

The fungus forms spots which are numerous and become brown to ashen grey in colour. The conidiophores come out from well developed stromata in fascicles, are pale brown, not branched, septate, geniculate and measure 4 x 25 – 115μ. The conidia are hyaline, acicular, septate and measure 4 x 54 – 165μ.

The above species has already been reported from India, by Sydow and McRae (l.c.) on *Beta vulgaris* L.

*Cercospora eugeniae* (Rangel) Chupp, A monograph of the fungus genus Cercospora, p. 406, 1954; Thirumalachar and Govindu, Sydowia 8 : 344, 1954.

On living leaves of *Syzygium cuminii* Skeels (Myrtaceae), Gurdaspur (Punjab), 22–11–1959, R. L. Munjal, H.C.I.O., No. 27092.

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* Previous papers in this series have been published in Indian Phytopath. I & II in vol. 12 (1), 1959; III in vol. 12 (2), 1959; IV in vol. 13 (2) 1960.

The fungus produces irregular, large dark purple spots, bearing fructifications on both sides of the leaf. The conidiophores arise in fascicles from stromata, are pale olivaceous brown, septate, not branched, not geniculate, short and measure 3 – 5 x 9 – 37μ. The conidia are pale olivaceous obclavate – cylindric, septate and measure 2 – 3x19 – 81μ.

Thirumalachar and Govindu (l.c.) reported this species from India on *Eugenia* sp.

*Cercospora geranii* Kellerm. & Swingle, Journ. Mycol., **5** : 74 : 1889.

On living leaves of *Geranium* sp. (Geraniaceae), Kulu, Kangra (Punjab), 18–11–1959, H. S. Gill and Gian Singh, H.C.I.O., No. 27052.

The fungus forms spots which are reddish brown, having fructifications on both surfaces of the leaf. The conidiophores arise from stromata in dense fascicles, are pale olivaceous, simple and measure 3.5 – 5 x 32 – 50μ. The conidia are hyaline to subhyaline, narrowly obclavate, septate and measure 2.0 – 3.5 x 29 – 61μ.

*Cercospora gossypii* sp. nov.

Foliorum maculae circulares, 1 – 2 mm diam., griseo albidae in medio, margine angusto rotundo fusce brunneo, frutificationibus vulgo epiphyllis stroma parvum, fusce brunneum, subglobosum, usque 31μ diam; fasciculi patentes, densi; conidiophori pallide vel olivaceo-brunnei, apicibus dilute colcratis, sparse geniculati, septati, nonnumquam constricti septa, non ramosi, latitudine irregulares, 4 – 6 x 15.5 – 77.5μ; conidia pallida, obclavata, minora cylindrica, septata, recta vel curvata, basi obconice truncata, apice subacuto vel obtuso, 3 – 4 x 12 – 62μ.

In foliis viventibus *Gossypii hirsuti* Linn. e fam. Malvacearum., I.A.R.I. New Delhi (Delhi), die 30 decembris anni 1959 a Ved Prakash H.C.I.O., No. 27095 Typus.

Leaf spots circular, 1 – 2 mm. in diameter, greyish white in centre with a narrow, raised, dark brown margin; fruiting mostly epiphyllous; stromata small, dark brown, subglobose, upto 31μ in diameter; fascicles spreading, dense; conidiophores pale to olivaceous brown, tip dilutely coloured, spraingly geniculate, septate, sometimes constricted at septa, not branched, irregular in width, spore scar prominent, 4 – 6 x 15.5 – 77.5μ; conidia pale, obclavate smaller cylindric, septate, straight to curved, base obconically truncate, tip subacute to obtuse, 3 – 4 x 12 – 62μ.

On living leaves of *Gossypium hirsutum* L. (Malvaceae), I.A.R.I., New Delhi (Delhi), 30–12–1959, Ved Prakash, H.C.I.O., No. 27095.

*Cercospora helianthicola* Chupp & Viegas, Bol. da Soc. Brasil de Agron. **8** : 29, 1945.

On living leaves of *Helianthus annuus* L. (Compositae), I.A.R.I., New Delhi (Delhi), 4–8–1960; Girdhari Lall, H.C.I.O. No. 27093.

The fungus forms spots which are dark brown, bearing fructifications on both surfaces of the leaf. The conidiophores arise from small stromata in dense fascicles, are pale brown, geniculate, septate very rarely branched, and measure 3 – 5 x 22 – 90μ. The conidia are hyaline, acicular, septate and measure 2.0 – 3.6 x 8 – 140μ.

*Cercospora lathyri—aphacae* sp. nov.

Foliorum maculae indefinitae, fructificationibus amphigenis, effusae, elongatae, ad nervos maiores evolutae, confluentes, nonnumquam totam superficien operientes, fuliginosae; stromata nulla vel nonnulis cellulis brunneis constantia, fasciculi rari vel densi; conidiophori pallidi, recti vel tenuiter curvati, non ramosi, non septati, 0 – 1 – -geniculati, 5 – 6 x 12 – 56 μ; conidia obclavata, pallida, recta vel aliquantum curvata, 0–5–septa, basi longa obconice truncata, spice obtuso, 5 – 6 x 22 – 58μ.

In foliis viventibus *Lathyri aphacae* L. (Leguminosae) in Vegetable Breeding Station, ad Katrain, in Kangra (Punjab) 13.5.1960, Girdhari Lal, H.C.I.O. No. 27096 Typus.

Leaf spots indefinite, fruiting amphigenous effuse, elongate, running along the major veins, confluent, sometimes the whole surface covered, sooty in colour; stromata lacking or a few brown cells; fasicles few to dense; conidiophores pale, straight to slightly curved, not branched, not septate, 0 – 1 geniculate, 5 – 6 x 12 x 12 – 56μ; conidia obclavate, pale, straight to somewhat curved, 0 – 5 septate, base long obconically truncate, tip obtuse, 5 – 6 x 22 – 58μ.

On living leaves of *Lathyrus aphaca* L. (Leguminosae), Vegetable Breeding Sub-station, Katrain, Kangra (Panjab), 13–5–60, Girdhari Lal, H.C.I.O., No. 27096.

*Cercospora occidentalis* Cooke, Hedwigia, **17** : 39, 1878; Sacc. **4**: 463, 1886; Sydow and McRae, Ann. Crypt. Exot. **2** : 267, 1930.

On living leaves of *Cassia tora* L. (Leguminosae), Mandi (H.P.) 8–11–1959, H. S. Gill and Gian Singh, H.C.I.O. No 27048.

The fungus forms, fructifications in effuse patches on both surfaces of the leaf. The conidiophores are borne in dense fascicles which are pale to medium brown, sparingly septate, sometimes geniculate, and measure 5 – 6 x 43 – 65μ. The conidia are olivaceous, cylindro-obclavate, multiseptate and measure 3.5 – 4 x 43 – 68μ.

This fungus has already been reported from India by Sydow and McRae (l.c.) on *Cassia occidentalis* L.

*Cercospora oplismeni* sp. nov.

Foliorum maculae lineares, nonnumquam coalescentes, 1 – 5 mm. longae, alutaceae, fructificationibus amphigenis; stromata subglobosa fusce brunnea, usque ad 46.0μ. diam; fasciculi rari vel densi; conidiophori

pallide olivaceo-brunnei, non ramosi, breves, septati, sparse geniculati, latitudine irregulares, sporarum cicatrice parve, 3 – 6 x 6 – 28μ; conidia hyaline, acicularia, recta vel curvata, multiseptata, apice acuto, 2 – 3 x 31 – 130μ.

In foliis viventibus *Oplismeni* sp. (Gramineae), Simla, (Punjab) 7-5-1960, Girdhari Lall, H.C.I.O. No. 27097 Typus.

Leaf spots linear, sometimes coalescing, 1 – 5 mm. in length, tan coloured, fruiting amphigenous; stromata subglobose, dark brown, up to 46μ in diameter; fascicles few to dense; conidiophores pale olivaceous brown, not branched short septate, sparingly geniculate, irregular in width, spore scar small, 3 – 6 x 6 – 28μ; conidia hyaline, acicular, straight to curved, multiseptate, base truncate, tip acute, 2 – 3 x 31 – 130μ.

On living leaves of *Oplismenus* sp. (Gramineae), Simla (Punjab), 7–5–1960, Girdhari Lall, H.C.I.O. No. 27097.

*Cercospora squalidula* Peck, N.Y. State Mus. Nat. Hist. Ann. Rept. **33** : 29, 1880.

On living leaves of *Clematis* sp. (Ranunculaceae), Mandi (H.P.), 8–11–1959, H.S. Gill and Gian Singh, H.C.I.O. No. 27053.

The fungus forms spots which are greyish in centre with purple border, having fructifications on both surfaces of the leaf. The conidiophores arise from stromata in dense fascicles, are pale to medium dark brown, not branched, geniculate, and measures 4 –5 x 50 – 90μ. The conidia are pale olivaceous, obclavato-cylindric, 3.5 – 5 x 58 – 180μ.

*Cercospora stachytarphetae* Ell. & Ev., Missouri Bot. Gard. Ann. Rept. **9** : 120, 1898; Sacc. **16** : 1070, 1902.

On living leaves of *Stachytarpheta indica* Vahl. (Verbenaceae), I.A.R.I., New Delhi (Delhi), 10–2–1960, Girdhari Lal, H.C.I.O. No. 27091.

The fungus forms spots which are gray in colour, bearing fructifications on both surfaces of the leaf. The conidiophors are pale to dark brown, in fascicles, septate, not branched, geniculate, and measure 4 – 6 x 29 – 186μ. The conidia are hyaline, acicular to obclavate, multiseptate, 3 – 4 x 22–109μ.

*Cercospora wellesiana* (Welles) Chupp, (l.c.) p. 427, 1954.

On living leaves of *Averrhoa carambola* L. (Oxalidaceae), Saharanpur (U.P.), 22–12–1953, S. P. Raychaudhuri, H.C.I.O. No. 27094.

The fungus forms spots which are pale to dark brown in colour, bearing fructifications mostly on the upper surface of the leaf. The conidiophores arise from stromata in fasciales, are very pale olivaceous brown, simple and measure 3.5 x 25 – 80μ. The conidia are hyaline, cylindric to very narrowly obclavate, septate, and measure 2 – 3.5 x 30 – 50μ.

Mohanty and Mohanty (Proc. Indian Sci. Cong. Assoc. Madras, **45** (3): 465, 1958) reported *Cercospora averrhoae* Petch, on this host from Orissa.

Sincere thanks are due to Dr. R. S. Vasudeva, Head of the Division, for his keen interest and encouragement thoughout these studies. We are also indebted to Dr. B. L. Chona, for his valuable guidance and Rev. Fr. Dr. H. Santapau, Head of the Department of Biology, St. Xavier's College, Bombay for rendering latin diagnosis of the new species.

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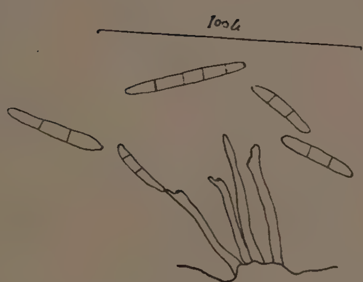


Fig. 2. *Cercospora lathyri aphacae*

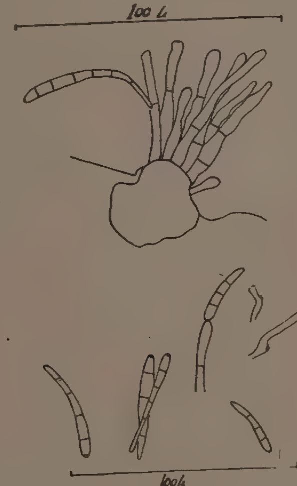


Fig. 1. *Cercospora gossypii*

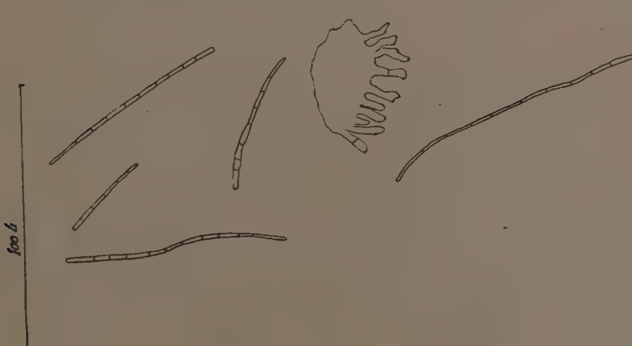


Fig. 3. *Cercospora oplismeni*

# GENUS TRETOPILEUS IN INDIA

R. L. Munjal

(Accepted for publication July 30, 1961)

Dodge* (1946) described a curious looking little fungus on Opuntia from Florida, which he named as *Tretopileus opuntiae*. This is the only report of this genus in literature so far.

In India fungi belonging to this genus have been encountered on a number of hosts e.g. *Thevetia nerifolia*, *Clerodendron* sp., *Malus sylvestris*, *Morus alba*, *Mangifera indica* and *Diffenbeckia* sp. from widely separated areas. These fungi were found growing on dead or drying twigs and appear to be saprophytic or at best weekly parasitic. The minute size and saprophytic habit of the fungus are perhaps the chief reasons of these having been rarely reported and careful search is likely to show their occurrence over large areas in tropical countries. The fungus was observed on *Thevetia* twigs throughout the year at Delhi, though it sporulates profusely after the summer rains in August and September. Invariably the twigs showing infection by this fungus also showed mixed growth of a number of other saprophytic fungi. The distinctive features of this group of fungi are the production of segmented stalk and a disc like spore with semi-translucent pore like markings on the upper surface.

The colonies are grey to smoky grey in colour, hairy, very variable in size on the twigs; the individual fructification giving an umbrella like appearance when disc like spore is still attached to the stalk. The substrate is often blackened. The mycelium which is embedded in the substratum is composed of hyaline, septate hyphae which are both inter and intracellular and ramify the host tissues. The hyphae aggregate to form stroma, which is innate-erumpent and from which one to many stalks (upto 30 seen) may arise. The stalk is composed of a bundle of septate hyphae, the outer ones being dark brown in colour and the inner hyaline. These are broad at the base and taper towards the apex which bears a round disc. The mature disc is thrown off and the stalk cells proliferate again to form another disc. Since the disc is much broader than the stalk, the stalk hyphae are some what spreading at the point of attachment with the disc, which later appear on the stalk as nodes or bands. One to several such nodes may be observed on a single stalk. Often the growth of stalk cells is quicker at the apex with the result that the outer cells of stalk a little down below do not develop their normal dark brown colour but remain subhyaline or light brown, which appear as light coloured patches on the stalk. The disc is round, convexo-concave, dark brown with poroid semi-translucent markings at its distal end. When an immature disc was removed from the stalk, it showed tearing away effect. The outer darker coloured hyphae forming a continuation with the outer cells of the disc and the inner hyaline ones forming the core of the disc. The inner hyaline

*Dodge, B. O. (1946) - *Bull. Torrey Bot. Club* Vol. 73, pp. 230 - 234

hyphae at the apex become rounded, which appear to be responsible for the period markings on the upper surface as also structure of the core of the disc. These cells were referred by Dodge as rows of four cells. Dodge was further of the opinion that these translucent poroid markings are probably a provision for the emergence of germ tubes or are places for sifting out of some spores that may be produced within the disc and therefore interpretted the disc as spore body. During the course of germination studies of several hundered discs, neither germ tubes were observed to emerge from these poroid markings nor any small spores formed within the disc. This disc was always observed to serve as a unit, which may be more correctly called a spore. The structural development here described or interpreted changes the entire concept of the fructification as envisaged by Dodge and should be considered to belong to Hyphomycetes rather than Basidiomycetes.

The fungus grew readily on Oat meal agar or Potato Dextrose agar at 25–27°C but typical stalks were rarely observed. Only once, a disc like body started developing on the tip of stalk but it did not grow any further. The mycelium was hyaline creeping with slight aerial hyphae. The stalk formation started after about 30 days' growth, when the mycelium became olivaceous and dark coloured later supporting 2–3 stalks. The cultural studies were made only of an isolate from *Thevetia*.

Some morphological differences were observed in the size and colour of stalk and disc in different collections but as the material in some collections is scanty, their specific determination is deferred for the time being and only *Tretopileus indicus* is proposed as a new species, which has been collected consistently for quite sometime on *Thevetia neriifolia* and on dead twig of an unidentified tree. The discs in this species are much bigger than *T. opuntiae* Dodge, which is reported to have disc measuring from 50 to 80μ.

*Tretopileus indicus* sp. nov.

Fructificationes efformatae in maculis ambitus indefiniti, mycelium vegetativum subcuticulare hyalinum vel tenuiter olivaceum, multiseptatum, 2.5 – 3.0 μ diam., postea efformans stroma erumpens cui insidunt 3 – 25 stipes. Stipes erectus vel paulum curvatus, fusce brunneus sed pallidioribus maculis quibusdam ornatus, 400 – 700μ longus, latior ad basin (60 – 75μ), angustus supra (35 – 40μ ad apicem), segmentis 1 – 9 constans, compositus fasciculis hypharum, quarum exteriores fusce brunneae, interiores vero hyaline, longitudinaliter dispositae, ad apicem pileo unico disciformo. Discus 130 – 175 x 130 – 160μ, nonnumquam stricte rotundus, 40 – 80μ altus, concavo-convexus, magnitud. ca. 50μ ad apicem concavum, fusce brunneus, durus, chitinosus poris multis vel spatiis pallidis (4 – 5μ) in latere convexo; ad maturitatem sat faciliter decidens ex stipe. In ramis emortuis vel marcescentibus *Thevetiae neriifoliae* ad Indian Agricultural Research Institute New Delhi, 12 octobris 1949, R. L. Munjal.

***Tretopileus indicus* sp. nov.**

Fructifications formed on discoloured spots of indefinite extent;

vegetative mycelium subcuticular hyaline to slightly olive coloured, multiseptate, 2.5–3.0μ in diameter, later forming an erumpent stroma which supports 3–25 stipes. Stipe erect or slightly curved, dark brown with lighter shades at places, 400 – 700μ in length, broader at the base (60 – 75μ), narrow above (35 – 40μ at the tip), 1–9 segments or jointed, composed of a bundle of hyphae, outer ones dark brown, inner ones hyaline, arranged length wise. Stalk bearing a single pileus like disc at the tip. Disc 130–175 x 130–160u, sometimes exactly round; 40–80μ concavo-convex, measuring about 50μ at the concave end, dark brown, tough, chitinous with many little pores or light coloured spaces (4–5μ) on the convex side, mature discs easily separating from the stalk.

On dead or dying twigs of *Thevetia neriifolia*, Indian Agricultural Research Institute New Delhi 12–10–1949, R. L. Munjal.

Sincere thanks are due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology for his keen interest, valuable suggestions and encouragement. I am indebted to Rev. Fr. Dr. H. Santapau for latin diagnosis of the new species.

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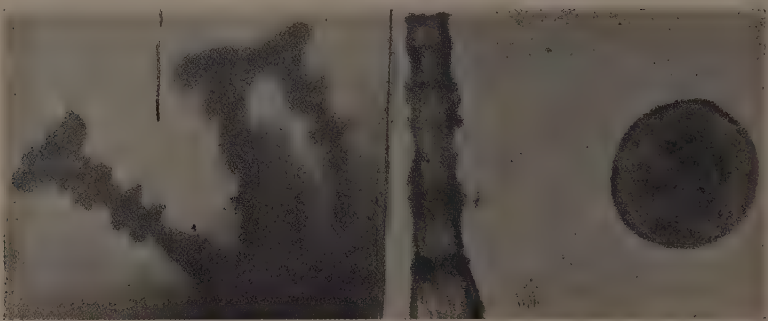
B A



*Tretopileus indicus*

A. Microphotograph showing stipe and the disc.

B. Microphotograph showing discs attached to the stipes.

# MOSAIC DISEASE OF ZINNIA ELEGANS JACQ.

R. N. PRASAD AND S. P. RAYCHAUDHURI

(Accepted for publication August 10, 1961)

A mosaic disease of *Zinnia elegans* Jacq. characterised by light and dark green mottling of the foliage accompanied by vein-clearing and green vein-banding (Fig. 1) was observed at Delhi in 1955. The affected plants are neither appreciably stunted nor the leaf size is reduced. The flowers appear normal but are fewer than those on the healthy plants. The diseased plants are pale in appearance. The symptoms suggested the disease to be of virus origin. Experiments were, therefore, undertaken to study the mode of transmission, host range and physical properties with a view to identify the causal virus.

MATERIAL AND METHODS: The inoculum for the experimental work was obtained from mosaic affected zinnia plants in the field and multiplied in tobacco (*Nicotiana tabacum* L. var. Harrison Special) as the seedlings of the latter were readily available in large quantities for experimental work. All the experiments were, therefore, conducted using tobacco as the test plant. Standard extract prepared by crushing washed and blotted leaves and adding 1 ml. of distilled water for every gm. of material, was used for all inoculations, except where otherwise mentioned. For the insect transmission work, the insects were collected from the field at the Indian Agricultural Research Institute and colonies of virus-free insects were established on healthy plants.

EXPERIMENTAL: TRANSMISSION OF THE VIRUS: The virus was found to be readily transmissible by mechanical inoculation by the leaf rubbing method but was not transmitted through the seed obtained from the diseased zinnia plants. Out of four species of aphids tested for the insect transmission of the virus, *Aphis gossypii* Glov. and *Myzus persicae* Sulz. could transmit the virus when released on healthy tobacco plants after feeding them on the diseased ones. All attempts to transmit the virus through *Lipaphis erysimi* Kalt (*Rhopalosiphum pseudobrassicae* Davis) and *Aphis craccivora* Koch. (*Aphis laburni* Kalt) were unsuccessful.

HOST RANGE: Out of the 54 species and varieties of plants belonging to 13 different families tested, the virus could be successfully transmitted only to *Nicotiana tabacum* L. vars. Harrison Special and White Burley, *N. glutinosa* L., *Petunia hybrida* Vilm., *Cucumis sativus* L. and *Momordica charantia* L. The virus induced blistering and mottling of the leaves accompanied by reduction in leaf lamina resulting in filiformy in *Nicotiana glutinosa* L. (Fig. 2) and pronounced vien-clearing accompanied by dark and light green areas and slight wrinkling and puckering of the leaf surface in *Cucumis sativus* L. (Fig. 3). *Nicotiana tabacum* L. and *Petunia hybrida* Vilm. showed characteristic light green or chlorotic patches more often on the tips and margins of the leaves (Figs. 4 and 5) but often the chlorosis

1 2

3 4

Fig. 1. Symptoms of mosaic disease on *Zinnia elegana* Jacq.
Fig. 2. Symptoms developed on *Nicotiana glutinosa* L.
Fig. 3. Symptoms developed on *Cucumis sativus* L.
Fig. 4. Symptoms developed on *Nicotiana tabacum* L.

5 6

Fig. 5. Symptoms developed on *Petunia hybrida* Vilm.
Fig. 6. Symptoms developed on *Momordica charantica* L.

extended to the rest of the leaf lamina. Slight vein-clearing followed by irregular mosaic mottling of the leaves characterised the symptoms on *Momordica charantia* L. (Fig. 6).

The other inoculated plants which remained unaffected and also did not prove to be symptomless carriers include the following:—

*Lycopersicon esculentum* Mill., *Capsicum frutescens* L., *Datura stramonium* L., *Solanum nigrum* L., *S. nodiflorum* Jacq., *S. melongena* L., *Pisum sativum* L., *Vigna sinensis* Savi, *Cajanus cajan* (L.) Millsp., *Crotalaria juncea* L., *Dolichos lablab* L., *Vicia faba* L., *Cyamopsis tetragonoloba* (L.) Taub., *Lagenaria siceraria* Standl., *Luffa aegyptiaca* Mill., *Citrullus vulgaris* Schrad., *Cucumis melo* L., *Cucurbita moschata* Duchesne, *Tagetes patula* L., *Lactuca sativa* L., *Calendula officinalis* L., *Carthamus tinctorius* L., *Helianthus annuus* L., *Cosmos* sp., *Chrysanthemum* sp., *Dahlia* sp., *Coreopsis* sp., *Brassica campestris* L., *B. oleracea* L. var. *capitata* L., *B. oleracea* L. var. *botrytis* L., *B. caulorapa* Pasq., *B. rapa* L., *Raphanus sativus* L., *Spinacia oleracea* L., *Kochia indica* Wight., *Portulaca* sp., *Dalphinium consolida* L., *Gomphrena globosa* L., *Amaranthus tricolor* L., *Daucus carota* L., *Abelmoschus esculentus* (L.) Moench., *Althaea rosea* L., *Sesamum orientale* L., and *Vinca rosea* L.

PROPERTIES OF THE VIRUS: The virus was found to tolerate exposure for 10 minutes to a temperature of 50°C., but not to 53°C. The dilution-end-point of the virus was found to lie between 1 : 100 and 1 : 200 and the virus retained its infectivity upto 16 hours at room temperature (10°–18°C.). When stored at 7–9°C. in a frigidaire the virus could withstand storage for 5 days but not for 6 days. The pH stability of the virus was found to range between 4.00 and 10.00 when inoculations were made immediately after extracting juice. But after 10 hours storage at 7–9°C. and 21–31°C. the virus could withstand a pH range of 5.00 to 9.00 and 6.00 to 8.2, respectively. The virus in the undiluted extract as well as in standard extract could tolerate exposure to ultraviolet irradiation upto 1 hour but was inactivated after 2 hours. At dilutions of 1 : 10 and 1 : 50, however, the virus lost its infectivity after an exposure of 30 and 15 minutes, respectively. Chilli juice was found to inhibit the virus completely when mixed with the virus extract in the proportion of 1 : 2 and mixture stored for 5 minutes at 28°C.

DISCUSSION: Elmer (1925) reported a mosaic disease of zinnia transmissible to tomato. Noble (1954), while giving a summary of the plant diseases recorded from New South Wales for the season 1932-33 also reported the occurrence of mosaic disease of zinnia. The mosaic virus reported herein differs from all other viruses known to infect zinnia in host range, properties or the mode of transmission, excepting *Cucumis* virus 1 (Doolittle, 1920) which resembles the virus now reported in some of the properties, while the insect vectors are the same. *Marmor cucumeris* var. *judicis* Holmes has been reported to induce formation of necrotic local lesions in zinnia (Price, 1935). It, therefore, appears that the virus causing zinnia mosaic reported herein is a strain of *Cucumis* virus 1 and it is proposed to designate it as *Marmor cucumeris* var. *zinniae* var. nov. according to Holme's (1948) system of classification.

### Summary

A mosaic disease of *Zinnia elegans* Jacq. occurring in Delhi has been found to be sap transmissible. The causal virus is transmitted by *Aphis gossypii* Glov. and *Myzus persicae* Sulz. but not through the seed of infected zinnia plants. Out of 54 plant species and varieties belonging to 13 different families inoculated with the virus, only *Nicotiana tabacum* L., *N. glutinosa* L., *Petunia hybrida* Vilm., *Cucumis sativus* L., and *Momordica charantia* L., were infected producing systemic mosaic symptoms.

The virus has a thermal-death-point of 50–53 °C., dilution-end-point between 1 : 100 and 1 : 200 and longevity in *vitro* of 16–18 hours and 5-6 days at room temperature (10–18 °C) and 7–9 °C, respectively. The pH stability of the virus was found to range between pH 4 to 10.

The causal virus has been identified as a strain of *Cucumis* virus 1 and designated *Marmor cucumeris* var. *zinniae*.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# MOLYA DISEASE OF WHEAT AND BARLEY IN RAJASTHAN

GOPAL SWARUP AND KISHAN SINGH

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INTRODUCTION: A serious disease of wheat and barley was first observed in some areas of Rajasthan during 1952. It is known locally as 'Molya' and causes heavy annual losses to wheat and barley crops. Though reliable estimates are not available yet it can be safely said that, in the affected areas, more than 50 per cent of the crop is lost due to this disease. The disease is far more serious in barley than in wheat. In fields where infestation is particularly severe losses amount to practically 100 per cent. The practice of the cultivators in Rajasthan is to take wheat and barley on the same land year after year mainly because these are staple crops and they can not afford to take any other crop. Inspite of heavy manuring the losses are heavy in the infested fields. According to local reports the disease appears to have been first observed in District Sikar—a place north-east of Jaipur and has gradually spread to Jaipur area. These two districts form the main areas where the disease is manifested year after year in a severe form. Investigation of this disease has been in progress in this Division for the last several years, with a view to ascertain the cause and also to suggest, if possible, suitable control measures. The experimental studies undertaken in this respect are reported herein.

SYMPTOMS OF THE DISEASE: The disease manifestes itself in the field in the form of stunted, pale looking plants. Such diseased plants do not grow beyond 1 to 2 ft. from the ground level. In a newly infested field the disease starts in small patches not exceeding 2 to 3 feet in diameter. The patch gradually increases every year with continuous cultivation until the whole field gets completely infested. In a badly infested field the whole crop remains stunted and practically no grain formation takes place. Tillering is also markedly reduced. In the young stage the diseased seedlings are pale yellowish green in colour, stunted with leaves comparatively thinner and narrower than the healthy ones. There is very little root formation and are smaller, under-developed and bunchy in places than healthy ones. The bunchy appearance of roots is due to formation of a number of small rootlets. As the infection advances slight swellings near the tips of roots can be easily seen. This condition is generally found within 4–6 weeks after sowing, that is, by the end of November or beginning of December. By the middle of February glistening white female bodies of nematodes can be seen adhering to the roots. A couple of weeks before the harvest time these white bodies tend to become brown in colour and may either remain attached to the roots or fall off in the soil after root decay. From 15 to 20 of these brown bodies (cysts) were obtained from a single affected plant which could be easily pulled out from soil.

MATERIAL AND METHODS: Diseased as well as healthy plants with roots and soil samples from the rhizosphere were collected from the infested

fields for experimental studies. Isolations from the roots of diseased and healthy plants were made on 2 per cent potato dextrose agar medium. Later the inoculum for the soil inoculations was raised on soil maize medium.

Soil samples were analysed for nemic population by the modified Baermann funnel technique (Christie and Perry, 1951). Two grades of sieves (60 and 325 meshes) were used for capturing the nematodes. The residue from the 325 mesh sieve was washed down in a beaker and then filtered through a funnel having a fine mercerised cloth on top and a rubber tube with pinch cock at the other end. It was then left overnight for the nematodes to come down and collect at the bottom of the funnel. Examination and population counts of the nematodes were done on the following day.

EXPERIMENTAL: Survey of the districts of Sikar, Jaipur and Ajmer was made and since the disease was equally well severe in Jaipur as at Sikar, soil samples along with the roots were collected mostly from affected fields in Jaipur district only. The samples were kept in alkathene bags at room temperature (15 to 20°C.). Isolations from the roots of diseased plants were made immediately after collection.

(a) Inoculation experiments with fungi isolated from diseased wheat and barley plants: Inspite of the fact that a parasitic nematode was found associated with the diseased plants, it was considered desirable to explore the possibilities of a fungus being the cause of the disease. With this aim in view a total number of 200 isolations were made from roots of affected plants. Isolations were made both at the initial stage of symptoms as well as at the advanced stage of disease. Only 3 genera of fungi viz. *Helminthosporium*, *Fusarium* and *Pythium* were represented in these isolations. All the isolates were tested individualy for pathogenicity and in none of the inoculated pots 'Molya' symptoms could be reproduced. In all probability these fungi establish after penetration by the nematode larvae.

(b) Role of *Heterodera avenae* (=*H. major*) in causing the disease: Prior to conducting any pathogenicity tests with the cysts and larvae of the nematode, the soil samples collected during January, 1959 and March, 1959 were analysed for population of *Heterodera*. The soil samples collected during 1958 and used in the experiment were also analysed before use. The data with reference to population of *Heterodera* are sét out in Table I.

As a result of this analysis seven samples out of the ten were selected for extraction of cysts. The samples selected were Nos. 1, 5, 6, 7, 8, 9 and 10. The cysts extracted from sample number 1 (collected in 1958) were kept separate from those collected from the other six samples. Cysts collected from the 1959 samples were mixed together.

(c) Inoculation experiments with cysts: From 1 to 25 cysts were put in 4" pots containing sterilized soil. Wheat (Agra local) and barley

TABLE I. Analysis of soil samples with regard to *Heterodera* population

| Sl. No. | District | Locality | Date of collection | Crop | Population of *Heterodera* | | | Apparent disease condition in field. |
|---|---|---|---|---|---|---|---|---|
| | | | | | Larvae | Male | Cysts | |
| 1. | Jaipur | Bhankorota | March, 1958 | Barley | — | — | 813 | Over 70 per cent |
| 2. | Sikar | Chala | Feb. 1958 | ,, | — | — | 81 | Below 10 per cent |
| 3. | Jaipur | Blalwal | Jan. 1959 | Barley | 227 | 389 | — | Over 70 per cent |
| 4. | Jaipur | ,, | ,, | Wheat | 142 | 109 | — | Between 15 to 20 per cent. |
| 5. | Jaipur | Chomu | March, 1959 | Barley | — | — | 373 | Over 70 per cent |
| 6. | Jaipur | Bairath | March, 1959 | ,, | — | — | 819 | Over 70 per cent |
| 7. | Jaipur | Gobindgarh | ,, | ,, | — | — | 1,005 | ,, ,, ,, |
| 8. | Jaipur | Nevta | ,, | ,, | — | — | 823 | ,, ,, ,, |
| 9. | Jaipur | Asampura | ,, | ,, | — | — | 912 | ,, ,, ,, |
| 10. | Jaipur | Sanganer | ,, | ,, | — | — | 1,527 | ,, ,, ,, |

(collected locally from Rajasthan) were sown in them. The pots were kept on greenhouse benches and observations were taken at the end of 2 months period. Ten seeds were put in each pot and percentage of disease was calculated on the number of seedlings showing typical disease symptoms. The data are provided in Table II.

TABLE II. Inoculation results with cysts of *Heterodera* obtained from samples collected during 1958 and 1959.

| Number of Cysts per pot | Percentage of infection | | | |
|---|---|---|---|---|
| | Cysts from 1958 samples | | Cysts from 1959 samples | |
| | Wheat | Barley | Wheat | Barley |
| No cysts | 0 | 0 | 0 | 0 |
| 1 ... | 0 | 10 | 40 | 40 |
| 2 ... | 0 | 0 | 40 | 50 |
| 5 ... | 10 | 0 | 80 | 90 |
| 10 ... | 10 | 0 | 100 | 100 |
| 15 ... | 10 | 10 | 100 | 100 |
| 25 ... | 10 | 10 | 100 | 100 |

The exaplanation for no infection or low infection in case of inoculations from 1958 collected cysts could only be the non-hatchability of the majority of the cysts used. At the time of inoculations cysts were selected at random without any bias. At the end of the experiment the soil of the inoculated pots which did not show any symptoms or showed only little, was again analysed and most of the cysts were recollected. These cysts when examined under the microscope showed black spots on the inside. On rupturing these, some blackened mass and some disintegrated material was released. Apparently the hatching did not take place because of the disintegration of contents. Again since good infection was obtained in case of cysts collected from 1959 samples, it appears that cysts start losing viability after a year if proper host is not available. Hatchability of cysts is known to be affected by drying (Winslow, 1954; Hesling, 1956) and it is possible that majority of the cysts obtained from 1958 soil samples had lost their viability since these samples were kept in the laboratory at room temperature (15 C to 40°C) throughout the two year period before use and by this time they had completely dried up.

(d) Inoculation experiments with larvae: Cysts were put for hatching in three media viz. water, wheat root diffusate and barley root diffusate. The diffusates were obtained in the manner described by Calam *et al* (1949) for potato. Ten cysts were put on each medium for hatching. The temperatures used for testing hatching were room temperature (22 – 23 °C), 20°C and 25°C. Hatching was observed within 48 hours and the maximum percentage (90 per cent) of hatched larvae was

obtained in the case of barley root diffusate at room temperature. At this temperature the percentage of hatching of the 1958 collected cysts varied from 5 to 30.

Individual larvae were picked out under the binocular for inoculation purposes, which was done by two methods. In the first case larvae were put in sterilized soil at the time of sowing of seeds. In the second case inoculations with larvae were made a week after sowing, just at the time when seedlings were emerging. Hand picked nematode larvae were put in 0 size gelatin capsules and inserted near the growing tips of the roots. The pots were maintained on glass house benches and the temperature during the experimental period ranged between 16°C to 25°C. Suitable controls were also maintained. Differences between the growth of inoculated and uninoculated seedlings were apparent within a month. The final observations on the growth and root development were made at the end of two month period. Figs. 1 and 2 show differences in growth and root-development in healthy and infected plants. The data are presented in Table- III.

A B

A B

Fig. 1. Showing differences in growth of healthy and infected plants.
A. Healthy plants. B. Infected plants

Fig. 2. Showing differences in the root development of healthy and infected plants.
A. Healthy plants. B. Infected plants.

It was observed that better infection was obtained when inoculations were made a week after sowing than at the time of sowing. It appears that some of the larvae put in the soil at the time of sowing may have starved out by the time first roots started coming out. It was observed that with the increase in number of larvae better percentage of infection was obtained. Further, more than 50 per cent infection was obtained when the number of larvae introduced in soil was from 15 to 20.

Discussion: These studies have confirmed that the serious disease of wheat and barley occurring in Rajasthan is caused by the cyst forming nematode belonging to the genus *Heterodera* as reported earlier (Vasudeva 1957 and 58). Our identification of the causal nematode agrees with that

recently reported by Prasad *et al* (1959). A similar disease of cereals. commonly called as cereal root eelworm, is already well known in European countries (Franklin, 1949). It is also known to attack oats and rye besides wheat and barley. In view of the large populations of this nematode found associated with the roots of the diseased plants and also the negative results obtained from the fungus isolations made from such roots, the question of any fungus being solely responsible for the disease does not seem possible. As the pathogenicity experiments have shown the nematode is the main cause for the disease. The part played by the associated micro-organisms reported earlier yet requires to be determined.

TABLE - III. Inoculation results with larvae of *Heterodera.*

| Number of larvae per pot | Percentage of infection when inoculated | | | |
|---|---|---|---|---|
| | Along with sowing | | One week after sowing | |
| | Wheat | Barley | Wheat | Barley |
| No larvae ... | 0 | 0 | 0 | 0 |
| 2 ,, ... | 0 | 0 | 0 | 10 |
| 5 ,, ... | 0 | 0 | 10 | 10 |
| 10 ,, ... | 10 | 10 | 20 | 20 |
| 15 ,, ... | 10 | 10 | 30 | 50 |
| 20 ,, ... | 40 | 50 | 80 | 90 |
| 25 ,, ... | 80 | 70 | 90 | 100 |
| 50 ,, ... | 100 | 100 | 100 | 100 |

*N.B*:—Ten seeds were sown in each pot and the infection percentages were calculated on the basis of number of seedlings showing stunted growth and whether or not cysts were formed on the roots of such plants. Only visual observations are recorded here.

The results of the inoculation tests made with the 1958 and 1959 collected cysts indicate that the viability of the majority of the one year old cysts is lost as a result of drying. Even if slight moisture is retained in the soil then larvae may hatch out and starve themselves to death in the absence of a host plant. Reduction in the hatching of cysts as a result of drying is another important factor and would be of significance from the point of view of control of the disease particularly by cultural practices and rotation.

Another important fact borne out from these investigations is that if the population is low and larvae hatch out prior to or just at the time of sowing, majority of them may die due to starvation and thus result in low infection. The possibility of hatching of larvae, prior to or at the time of sowing, under field conditions, can not be ruled out since temperature and moisture appear to play an important role in inducing cysts to hatch. As the cultivators in Rajasthan can not afford to follow suitable rotations

population of cysts continues to build up and even if some cysts hatch out prior to sowing, there are always enough left which hatch out at the proper time and bring about infection.

## Summary

A serious disease of wheat and barley occurring in Rajasthan has been described. Pathogenicity tests have confirmed the causal agent as the cyst forming nematode, *Heterodera avenae* (=*H. major*). When the inoculations are made a week after sowing infection obtained is much greater than when the inoculations are made at the time of sowing.

Viability of the one year old cysts of *Heterodera avenae* appeared to be adversely affected, when stored at room temperature.

Acknowledgement: The investigation was taken up at the suggestion of Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology to whom grateful thanks are due. His guidance and valuable criticism during different stages of work have been invaluable in the conduct of these experiments.

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# DEFICIENCY DISEASE OF GUAVA IN RAJASTHAN AND ITS CONTROL

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Vasudeva (1954), and Vasudeva and Raychaudhuri (1954) reported a serious disease of guava caused by zinc deficiency in Pushkar valley. The disease is characterised by severe reduction in leaf size, interveinal leaf chlorosis, suppression of growth and dieback of leaders. During 1954–56, extensive survey was carried out in Ajmer and certain parts of Jodhpur in order to determine the occurrence of the disease. Altogether, 14 orchards were examined in Ganera, Chamondia, Hatundi and Adarsnagar areas in Ajmer and at Mandore Government Agricultural Farm and Sukh Labera in Jodhpur. None of the orchards was found to be free from the disease and in some orchards in Pushkar valley in Ajmer, the incidence of the disease was 100 per cent. Experiments with a view to control the disease were carried out during 1954-56 and results are reported in this paper.

Experimental: Various chemicals pure and commercial were used for treatment of diseased guava plants in different orchards. The treatments included foliage spray, soil application and shoot and trunk injections.

Foliar spray: Preliminary spraying experiments were conducted in the first instance with various chemicals such as zinc sulphate (chemically pure) singly or in combination with cupric oxide, manganese sulphate, ferrous sulphate, copper sulphate, cupric oxide and Bordeaux mixture in two orchards. Within 4–6 weeks after spraying some beneficial effect was observed in the plants sprayed with zinc sulphate whereas no visible effect was observed in any of the other treatments.

Regular experiments on foliage spray of zinc sulphate were, therefore, carried out in four other orchards belonging to different cultivators. Two to five sprayings of zinc sulphate (Zinc sulphate 1.0 lb.; hydrated lime 0.7 lb.; water 16 gallons) were given at intervals of approximately 2–3 months depending on the age of the plants and intensity of the disease. Marked improvement of the diseased plants was noticed with the very first spray and the new growth on the affected branches showed healthy and normal leaves (Fig. 1). The subsequent sprayings helped to cure most of the plants and the new shoots were observed to bear normal flowers and fruits.

Later, experiments were carried out in some of the orchards using commercial zinc sulphate and 'Nu Z' (zinc oxysulphate), a neutral zinc compound containing 55 per cent metallic zinc, and a product of Tennessee

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Corporation, U.S.A. It was observed that both commercial zinc sulphate as well as 'Nu Z' are effective in curing the disease.

APPLICATION OF ZINC SULPHATE THROUGH SOIL: In order to determine the effect of the application of zinc sulphate through soil two experiments were laid out with young and old plants, respectively. The treatments were as follows:—

(i) application of zinc sulphate at the rate of 4 oz. per plant

(ii) application of zinc sulphate at the rate of 8 oz. ,,

(iii) application of zinc sulphate at the rate of 12 oz. ,, ,,

(iv) no application (Control).

Zinc sulphate was applied to the plants by mixing the required quantity of the chemical with upper 4–6″ soil at a distance of about 6–9″ around the trunk. The plants were immediately watered after the treatments. The irrigation was applied at regular intervals to allow the chemical to be dissolved and absorbed by the roots.

In the first experiment conducted at Ganera both pure and commercial zinc sulphate were used separately on three plants of a bout 2 years in age under each treatment. In the second experiment conducted at Hatundi, four guava trees of 15 years in age were treated under each treatment using commercial zinc sulphate only. In both the cases the chemical was applied twice at an interval of three months.

The beneficial effect of the soil application of zinc sulphate (pure and commercial) was observed in the first experiment in the form of healthy growth in the new shoots (Fig. 2) in all the treatments except control. However, no differences were observed in the test plants under different treatments. In the experiment with the old trees at Hatundi, no beneficial effect of soil application was indicated under any of the treatments. This showed that the beneficial effect of soil application of zinc sulphate is more apparent in young plants.

SHOOT INJECTION: For shoot injection, Roach's method (Roach, 1938) was employed in which the tips of young severely affected branches were cut and dipped in zinc sulphate (zinc sulphate 1 lb.; hydrated lime 0.7 lb.; water 16 gallons) contained in a tube. All the 12 shoots injected in this manner were found to show beneficial effect on the new growth (Fig. 3).

TRUNK INJECTION: Fifteen grains of zinc sulphate in powder form were injected in the main trunk of the diseased guava plants which were about 4 years old by boring holes with a metal drill, introducing the chemical and plugging the holes with corks. The wound was bandaged with sterilised adhesive plaster. Controls were maintained where no

zinc sulphate was added to the holes. It was observed that the first injection did not show any beneficial effect. A second dose of 15 grains of zinc sulphate was, therefore, applied after two months and this resulted in the growth of healthy leaves in the new shoots (Fig. 4). It was, however, noticed that the beneficial effect was localised and restricted to only those branches which were on the side of the trunk injected.

1

2

Fig. 1. Guava disease cured by foliage spray of zinc sulphate. The lower leaves are diseased and reduced in size and the new leaves are healthy and normal.

Fig. 2. Guava disease cured by soil application of commercial zinc sulphate.

3

4

Fig. 3. Guava disease cured by shoot injection with zinc sulphate.

Fig. 4. Guava disease cured by trunk injection with zinc sulphate.

CONCLUSIONS: The beneficial effects of foliar spray of the diseased guava plants with zinc sulphate in the cultivators' orchards confirm the previous finding of Vasudeva and Raychaudhuri (1954). Further, from the results of the experiments reported in this paper, it has also been proved that the disease can be cured readily by soil application of zinc sulphate as well as by shoot or trunk injections. The beneficial results due to foliage spray have been observed within 4–6 weeks and complete cure of the diseased plants could be achieved after a few spraying operations depending upon the age and size of plants and intensity of the disease. The beneficial effect of soil application of zinc sulphate is rather slow and also some of the chemical is likely to be washed away due to leaching as the soils in Ajmer are sandy. In addition, it necessitates frequent irrigations. As Ajmer happens to be a dry area and there is lack of irrigation facilities, the cultivators find it difficult to irrigate the orchards re-regularly after the application of zinc sulphate in the soil. The shoot and trunk injections of zinc sulphate have resulted in localised effect restricted to the injected shoot or the branches on the side of the injected trunk, respectively. It is, therefore, obvious that foliar spray is the most effective and practicable method in this region for combating the disease. On an average, it was observed that six to eight fully grown plants could be sprayed with 1 lb. of commercial zinc sulphate which is as effective as the chemically pure one, so that the cost of spraying each plant including the cost of lime will be about 0.12 nP only. Hence for spraying fully diseased plants thrice during a particular season the cost will work out at about 0.37 nP. By investing such an insignificant amount the cultivators can effect saving of approximately Rs. 45.00 which is estimated to be the loss due to the disease per plant per year (Vasudeva and Raychaudhuri, 1954).


## SUMMARY

1. It has been shown that the deficiency disease of guava in Pushkar valley can be controlled by spraying the affected plants with commercial zinc sulphate (zinc sulphate 1.0 lb.; hydrated lime 0.7 lb.; water 16 gallons). It has also been shown that the treatments recommended are economical.

2. Soil applications of zinc sulphate at the rate of 4 to 12 oz. per plant have shown beneficial effect in young diseased plants.

3. Shoot and trunk injections with zinc sulphate indicated localised beneficial effect restricted to the injected shoot or the branches on the side of the injected trunks, respectively.


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# COMPARATIVE STUDIES OF MACROPHOMA MANGIFERAE AND ITS ULTRA-VIOLET LIGHT INDUCED MUTANT

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Introductory: Hingorani, Sharma and Nirmal Jit Singh (1961) obtained a stable mutant of *Macrophoma mangiferae* Hingorani and Sharma, the causal organism of blight disease of mango, by exposing it to ultra-violet light. This mutant appeared to differ from the parent culture in certain characters, particularly in its ability to produce pycnidia quicker and in abundance as also in having comparatively smaller pycnidia and pycnidiospores. Detailed morphological, cultural and pathological studies of the two isolates were, therefore, made with a view to compare them. The present paper is the result of this study.

Materials and Methods: The authentic cultures of *Macrophoma mangiferae* and its mutant were obtained from the Indian Type Culture Collection and purified by single-spore technique (Rawlins, 1933) before use. Plants of *Mangifera indica* (varieties *Chowsa*, *Dasheri*, *Langra* and *Sundhuri*), *Eugenia jambolina*, *Eryobotrya japonica*, *Ficus carica*, *Vitis, vinifera*, *Citrus sinensis*, *Musa sapientum* and *Psidium guajava* for inoculation tests and host-range studies were secured from the Horticulture Division of I.A.R.I., while those of *Cajanus cajan*, *Nicotiana tabacum*, *Physalis peruviana* and *Zea mays* were raised from seed in the Division of Mycology and Plant Pathology.

The stock cultures were maintained on oatmeal agar. For comparative growth and sporulation studies, 22 different media, varying greatly in their composition, were used. These were oatmeal agar, maize-meal agar, mango-leaf-decoction agar, potato-dextrose agar, yeast-infusion agar, malt agar, Richards' synthetic agar, Czapeck's synthetic agar, Coon's medium, Elliott's agar, Radicicola agar (Diehl's medium), Crabill's medium, Horne and Mitter's medium, Leonian's medium,, glucose agar, modified Richards' synthetic agar, Mix's K.U. medium Brown's synthetic agar, Das Gupta's synthetic agar, Barne's agar, Nitimargi's standard agar and asparagin glucose agar. The methods of preparation for the first 15 media were taken from either Rawlins (1933) or Riker and Riker (1936), while the formulaes for the remaining seven media per litre are given below. Twenty grams of agar agar were used in all the solid media tried here.

1. Modified Richards' synthetic agar: $KH_2PO_4$ 5g.; $MgSO_4$.. $7H_2O$ 2.5 g.; $KNO_3$ 10 g.; $Fecl_3$ 0.02 g.; potato starch 10 g

2. Mix's K.U. medium: $KNO_3$ 0.5g.; $MgSO_4.7H_2O$ 0.75 g.; $KH_2PO_4$ 1.25 g.; dextrose 1 g.

3. Brown's synthetic agar: $K_3PO_4$ 1.25 g.; $MgSO_4$. $7H_2O$ 0.75 g.; asparagin 2 g.; glucose 2 g.

4. Das Gupta's standard agar: $MgSO_4$. $7H_2O$ 0.75 g.; $K_3PO_4$ 1.25 g.; asparagin 2 g.; glucose 2 g.; potato starch 10 g.

5. Barne's agar: $K_3PO_4$ 1 g.; $NH_4NO_3$ 1 g.; $KNO_3$ 1 g; glucose 1 g.

6. Nitimargi's standard agar: $MgSO_4.7H_20$ 0.75 g.; $K_3PO_4$ 1.25 g.; asparagin 2 g.; glucose 2 g.

7. Asparagin glucose agar; asparagin 2 g.; glucose 20 g.

EXPERIMENTAL: (a) EFFECT OF DIFFERENT MEDIA ON GROWTH AND SPORULATION: In preliminary studies, it had been observed that the parent culture normally produced pycnidia after 40 days of growth, while the mutant strain developed them after 7 days. Therefore, colony characters and density of pycnidial formation were recorded after 40 days of growth while growth rate was measured on the sixth day when maximum differences were observed. The inoculated Petri plates of uniform size (10 cm.) were incubated at 30°C. $\pm$ 1°C. throughout this study.

No difference was observed in the growth rate of the two isolates on any of the media tried. The maximum growth rate (100 mm.) was obtained on Crabill's medium, mango-leaf-decoction agar, modified Richards' synthetic agar, potato–dextrose agar and oatmeal agar, whereas the minimum (56 mm.) was on glucose agar.

The two isolates behaved somewhat differently in colony characters on the various media tried. It was, however, difficult to separate them on the basis of these differences. In general, colour of the colonies varied considerably. Aerial mycelium of the mutant, wherever produced, was either fluffy or felty and sometimes stranded as on Das Gupta's standard agar or crustaceous as on Richard's synthetic agar. The parent isolate, on the other hand, produced profuse mycelium in majority of cases. The colonies were either circular or irregular, and zonation was prominent with mutant isolate.

The mutant formed pycnidia on all the media, except Radicicola agar and glucose agar, within 7 days, whereas the parent culture did not produce them on any of the media tried within that period. After 40 days, however, the parent isolate also produced pycnidia on some of the media, but in many cases they were found to be immature. On yeast extract agar, Mix's K.U. medium and Brown's synthetic agar, the mutant produced very few mature pycnidia. Fig. 1 shows pycnidial formation by the two isolates on 3 media. On Potato dextrose agar, the parent isolate produced pycnidia in moderate numbers and the mutant in abundance; on Elliott's medium, parent produced a few of them, whereas the mutant formed them abundantly; and on Brown's medium, the parent produced none, whereas the mutant developed a few.

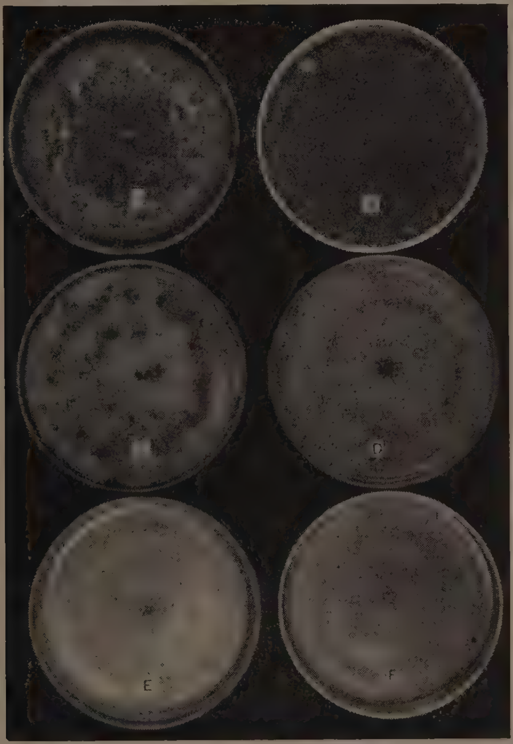


Fig. I. Formation of pycnidia by *Macrophoma mangiferae* and its mutant on different media.

A ... Parent } B ... Mutant } Potato Dextrose agar — C ... Parent } D ... Mutant } Elliot's agar

E ... Parent } F ... Mutant } Brown's Synthetic agar.

(b) Effect of depth of medium on growth and sporulation: The isolates of *Macrophoma mangiferae* and its mutant were grown on different quantities of Elliott's medium in Petri Plates (10 cm.) to determine the effect of depth of medium on the growth and pycnidial formation. The Petri plates were then incubated at 30°C±1°C. and the growth measurements and pycnidial formation were recorded after 6 and 40 days, respectively.

It was seen that depth of the medium had practically no differential effect on the growth rate of the two isolates. The mutant, however, formed

abundant pycnidia with 25 and 35 c.c. of the medium, but not with 15, 20 and 45 c.c. No pycnidia were formed when smaller quantities of the medium were used. No differences in the colony characters of the two isolates were seen.

(c) Effect of temperature on growth and sporulation: The two isolates were grown at different temperatures to study their temperature relationship. Elliott's medium and mango-leaf-decoction agar were used for the study. The cultures were grown in triplicate on these media, and incubated at different temperatures. The radial growth measurements were taken after six days of inoculation. The observations were also made on the pycnidial formation.

There was no difference in the minimum, optimum and maximum temperature requirements of the two isolates. Almost identical growth was obtained at different temperatures on the two media. Minimum temperature was somewhere between 5° and 10°C. as some growth was obtained at 10°C, after 10 days, but none at 5°C. and 40°C. Optimum temperature was between 25°C. and 30°C. There was no significant effect of temperature on the pycnidial formation by the two isolates.

(d) Effect of sunlight on growth and sporulation: Three sets of Petri plates with three replicates in each were arranged to study the effect of sunlight on the growth and sporulation of the two isolates The media used were Elliott's synthetic agar and oatmeal agar. One set was continuously kept in darkness by wrapping black paper around the bell jar; another was kept in complete darkness for 24 hours and then exposed to sunlight for the day-length; and the third was left near a window. All the three sets were kept on the same table so that there was no fluctuation in temperature.

It was seen that there was no difference in the growth and sporulation of the two isolates under different light conditions as growth rate and sporulation were normal in all the three cases. Mutant continued to form pycnidia within 7 days under all light conditions, whereas the parent isolate took 40 days to produce mature pycnidia. Furthermore, there was no difference in the density of the pycnidial formation.

(e) Effect of Hyderogen-ion concentration on growth and sporulation: The object of the experiment was to find out whether there was any difference in the growth rate and pycnidial formation of the two isolates at different ranges of pH. Elliott's medium was adjusted to different pH ranges by adding N/10 sodium hydroxide or hydrochloric acid. Three replications were kept in each case and the Petri plates were incubated at 30°C. $\pm$ 1°C.

It was clearly seen that both the isolates had a fairly wide range of toleration of hydrogen-ion concentration of the substrate as they could grow between pH 3.0 to 7.6. However, the optimum growth of the colonies was observed between pH 5.4 to 6.5 with both the isolates. No

growth was observed at pH 2.5 and 8.0. Finally, pH did not seem to have any effect on the pycnidial formation by the two isolates.

(f) EFFECT OF DIFFERENT INGREDIENTS OF ELLIOTT'S MEDIUM ON GROWTH AND SPORULATION: With a view to find out the basic requirements of different forms of nutrients for the optimum growth and pycnidial formation of the two isolates, Elliott's medium was used along with the media in which one of its components was eliminated. These media, six in all, were then inoculated in triplicate sets and incubated at 30°C.±1°C. The pH of all the media was adjusted to 6.5. After six days' growth, diameter of the colonies was measured. The observations regarding the colony characters and pycnidial formation after 40 days were also recorded.

Data obtained clearly showed that absence of dextrose in the standard medium considerably affected growth rate of the two isolates. No pycnidia were produced by the two isolates under these conditions even after 40 days. Absence of $KH_2Po_4$ in an otherwise complete medium resulted in no pycnidial formation by the parent. In this respect, the two isolates differed from one another. The mutant isolate further showed a tendency to produce pycnidia in aggregate, whereas this character was absent in the parent isolate. There were no differences in the colony characters of the two isolates.

Type of the phosphate used in the medium seemed to have considerable effect on the pycnidial formation. Maximum number of pycnidia were formed when $KH_2Po_4$ was added to the medium; intermediate with $K_2HPo_4$; and minimum with $K_3Po_4$.

(g) EFFECT OF DIFFERENT CARBON SOURCES ON GROWTH AND SPORULATION: In an earlier experiment, it was found that dextrose was essential for good growth and sporulation of the two isolates. It seemed desirable, therefore, to determine the effect of other carbohydrates in this respect. The carbohydrates used where galactose, laevulose, dextrose, sucrose, lactose and maltose. The carbohydrates were mixed separately at the rate of 2 per cent with the basic synthetic medium as suggested by Lilly and Barnett (1951).

The presence of a carbon source in the medium was found to be very essential for the normal development of the fungus as, in its absence, there was no growth and pycnidial formation. Of the carbon sources tried, dextrose was the best for mycelial growth and sucrose and maltose for the pycnidial production. Immature pycnidia were formed in the parent isolate on the medium containing either laevulose or dextrose, while no pycnidia were formed on the medium having galactose as its carbon source. Other carbon sources showed no influence on the development of the fungus.

Profuse and felty or fluffy aerial mycelium was produced by the parent isolate on these media, whereas the aerial mycelium of the mutant isolate was generally poorly developed. Colonies were either regular or irregular with compact mycelial growth. No zonation was observed in

the parent isolate, whereas mutant isolate showed some zonation on these media. Pycnidia of the mutant oozed out clear watery solution in the presence of lactose, and comparatively smaller spores were produced on the medium containing galactose as the carbon source. The size of the pycnidia was very much reduced on the medium containing sucrose in case of the mutant.

(h) Effect of different nitrogen sources on growth and sporulation: In order to study the utility of various nitrogen compounds for the growth and pycnidial formation of the parent and mutant isolates, different nitrogen compounds were incorporated in the basic synthetic medium as used for carbohydrate studies with this difference that the nitrogen source, *i.e.*, asparagin, was eliminated, and 2 per cent sucrose was incorporated instead. The nitrogen sources used were asparagin, ammonium tartrate, ammonium nitrate and potassium nitrate. These were used at the rate of 0.2 per cent. The Petri plates were inoculated in triplicate and incubated at a temperature of 30°C $\pm$ 1°C. The pH of all the media was adjusted to 6.5. After six days' growth, diameter of the colonies was measured. Observations were also recorded on the pycnidial formation and colony characters.

It was observed that asparagin was the best source of nitrogen for growth and sporulation of the two isolates. The parent culture formed immature pycnidia in the presence of ammonium nitrate and none in the absence of any nitrogen source. In case of the mutant, pycnidial size was greatly increased on the medium containing ammonium nitrate, while it was reduced in the presence of ammonium tartrate or potassium nitrate. The mycelial growth of the two isolates on these media was loose to compact and felty or fluffy.

(i) Morphological studies: Differences in the mycelial characters, pycnidia and pycnidiospores of the parent and the mutant isolates were studied on oatmeal agar.

The mycelium of both the isolates was whitish in colour when young, but later turned iron-gray in case of the parent and olive-gray in case of the mutant. The aerial mycelium was comparatively thinner than the submerged mycelium. The hyphae were irregular, septate and sparingly branched, which were at times rigid and slightly knotted. The branches originated below the septum. Due to anastomosing habit of the filaments, the hyphae formed strands, which were quite prominent in the mutant and less so in the parent. The hyphae of the two isolates measured 1.5 – 7.5$\mu$. Thus, there were no significant differences in the mycelial characters of the two isolates.

The pycnidia, when observed on mango leaves, looked like small pin heads, which were formed on the necrotic portion of the spot. The The pycnidia were formed in abundance in case of the mutant isolate and were comparatively smaller in size than those of the parent isolate. They were globose to sub-globose, light brown when young but steel brown at maturity, ostiolate, sub-epidermal, at first innate, then becoming erum-

pent. The pycnidia of the parent measured 77.0 – 232.0$\mu$ and those of the mutant 36.0 – 61.2$\mu$. Pycnidial wall was thick, 5 to 6 layered, consisting of pseudoparenchymatous cells.

The pycnidia were formed in the culture media after 40 days in case of the parent and after 7 days in case of the mutant. They first appeared as a congregation of mycelial filaments, which later became aggregated and formed small black specks enclosed in the loose hyphal wefts of the aerial mycelium on the surface of the medium. Pycnidia were steel, black, sclerotic with hard pycnidial wall and ostiolate. The ostiole appeared as a small depression. The size of the pycnidia in case of the parent ranged between 225–510 x 210–450$\mu$, while in case of the mutant isolate it was between 180–510 x 135 – 375$\mu$.

The pycnidiospores, as obtained from the host, were borne on small sporophores which were thin, slender, hyaline and slightly broader at the tip. The sporophores carried pycnidiospores at their tips singly. The spores were hyaline with thick granular protoplasm. They were unicellular, oblong to elliptical with both ends obtuse. They measured 10.5 - 24.5 x 5.3 – 7.0$\mu$ in case of the parent isolate, while in the mutant isolate the measurements were 9 – 15 x 3 – 6 $\mu$.

The pycnidiospores taken from a 6-week-old culture were similar to those obtained from the host except in their size. The pycnidiospores of the parent isolate measured 17.5 – 29.8 x 3.5 – 7.5$\mu$, while those of the mutant were 14.0 – 21.0 x 3.5 – 7.5$\mu$ in size.

The data are summarized in Table I.

TABLE I. Size of the hyphae, *pycnidia* and *pycnidiospores* of the parent and mutant isolates of *Macrophoma mangiferae*

| Structures | Size in microns | |
|---|---|---|
| | Parent | Mutant |
| Mycelial cells | 1.5–7.5 | 1.5–7.5 |
| *Pycnidia* | | |
| (a) Host | 77–232 | 36.0–61.2 |
| (b) Culture | 225–510 x 210–450 | 180–510 x 135–375 |
| *Pycnidiospores* | | |
| (a) Host | 10.5–24.5 x 5.3–7.0 | 9–15 x 3–6 |
| (b) Culture | 17.5–29.8 x 3.5–7.5 | 14.0–21.0 x 3.5–7.0 |

Thus, it will be seen that the parent culture and the mutant differ from one another in pycnidial and pycnidiospore size, the mutant having comparatively smaller pycnidia and pycnidiospores.

Spore germination of the two isolates was determined in distilled and tap water at different temperatures. Percentage of spore germination and length of the germ tubes were recorded. The two isolates germinated equally well in tap and distilled water. Temperature range at which spores of both the isolates germinated was the same (15° – 35°C.). At 30°C. (optimum temperature), spores started germinating after 2 to 3 hours and maximum germination was obtained after 5 hours. It, however, appeared that spores of the parent germinated in larger numbers and faster than the mutant. The pycnidiospores on germination gave out germ tubes either from one or both ends.

PATHOLOGICAL STUDIES: Young potted plants (2-months old) of mango varieties *Langra*, *Chowsa*, *Dasheri*, and *Sundhrui* were inoculated on both surfaces of the leaves as also on the stems with and without injury. Injury on the leaves was made by the pin-prick method, while on the stems half on inch long and 1 cm. deep cut was given with a sterilized blade. Proper controls were kept in each case. The average maximum and minimum temperatures recorded during the experimental period were 32°C. and 12°C., respectively.

Inoculation tests showed that infection could not be obtained without injury. Both the isolates infected all the four varieties of mango. However, the mutant appeared to be more virulent, as it produced abundant pycnidia on all the four varieties within a shorter period. Similar results were obtained with the stem inoculations. Control plants remained healthy throughout the experimental period.

Inoculations were also made on ripe and unripe fruits of mango variety *Dasheri* with injury after which they were kept in moist chambers for the rot to develop. Mutant rotted the whole fruit in four days, while the parent isolate produced only one third of the rot whithin the same period. Uninoculated fruits did not develop rot.

In addition to mango, *Eugenia jambolina*, *Eryobotrya japonica*, *Ficus carica*, *Vitis vinifera*, *Citrus sinensis*, *Musa sapientum*, *Psidium guajava*, *Physalis peruviana*, *Cajanus cajan*, *Nicotiana tabacum* and *Zea mays* were inoculated for host-range study. Inoculations were made after injuring leaves by the pin-prick method. Uninoculated plants were also kept in each case which served as controls.

The two isolates successfully infected *Eugenia jambolina*, *Eryobotrya japonica*, *Ficus*, *carica* and *Vitis vinifera*, thus showing no difference in their host-range. The mutant, however, appeared to be more virulent than the parent as it caused infection in 3 days and produced pycnidia in 5 days, while the corresponding figures for the parent isolate were 5 and 12 days. The number of pycnidia developed by the mutant was also comparatively greater. The control plants in each case remained healthy.

IV. DISCUSSION: Comparative studies between *Macrophoma mangiferae*, the causal fungus of blight disease of mango, and its ultra-violet light induced mutant have revealed that the two isolates differ from one

another in the following characters: (i) The mutant isolate produces mature pycnidia on majority of the media tried within 7 days, whereas the parent isolate develops them on a few media only and that also after 40 days; (ii) pycnidia and pycnidiospores of the mutant are comparatively smaller in size than those of the parent; (iii) The mutant appears to be more virulent than the parent as it produces pycnidia earlier and in abundance on all the hosts infected. This is further evidenced by the fact that the mutant isolate in comparison produces quicker rot of the mango fruits. There is, however, no difference in their host-range as both the isolates successfully infect the same hosts, namely, *Mangifera indica*, *Eugenia jambolina*, *Eryobotrya japonica*, *Ficus carica* and *Vitis vinifera*. A higher degree of virulence in case of the mutant may be explained on the basis of the increased inoculum produced by it, inspite of the fact that the parent culture shows higher percentage and faster spore germination.

The study of the basic requirements of different ingredients in Elliott's synthetic agar for growth and sporulation of the two isolates has given some interesting results. The absence of a carbohydrate source in the synthetic medium results in very little vegetative growth and no pycinidial formation in both the isolates. On the medium devoid of phosphate or nitrogen source, the pycnidial formation is either absent or immature pycnidia are formed in case of the parent isolate. Furthermore, it has been seen that potassium dihydrogen phosphate is the most suitable phosphate source for the pycnidial formation. Hafiz (1951) has, however, observed that $K_3Po_4$ is the most suitable source of phosphate for sporulation in *Ascochyta rabiei*.

While studying the development of the two isolates on different carbon sources, it has been found that dextrose is the best carbon source for mycelial growth and sucrose and maltose for pycnidial formation. Immature pycnidia are formed by the parent isolate when either laevulose or dextrose is added to the medium and no pycnidia are produced with galactose.

Amongst the nitrogen sources tried for the optimum growth and pycnidial formation by the two isolates, asparagin seems to yield the best results. However, the parent isolate produces immature pycnidia when ammonium nitrate is used as nitrogen source. In case of the mutant, an increase in the pycnidial size has been observed when ammonium nitrate is used, while the pycnidia remain smaller in size when ammonium tartrate or potassium nitrate is incorporated in the medium.

## V. Summary

(1) In these studies, *Macrophoma mangiferae* and its mutant have been compared. The mutant isolate produces mature pycnidia on majority of the media tried within 7 days, whereas the parent culture forms them on a few media within 40 days.

(2) Different sources of carbon and nitrogen as also the type of phosphate have been shown to affect the growth and pycnidial formation of the two islolates.

(3) There is no difference in the pH and temperature requirements of the two isolates.

(4) Pycnidia and pycnidiospores of the mutant are smaller than those of the parent culture.

(5) The two isolates successfully infect *Mangifera indica*, *Eugenia jambolina*, *Eryobotrya japonica*, *Ficus carica* and *Vitis vinifera*. The mutant isolate, however, appears to be more virulent as judged by the number of days required to produce the symptoms and the amount of rot produced on mango fruits by the two isolates.

The writers wish to record their grateful thanks to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology, for his keen interest and helpful suggestions.

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# NOTES ON INDIAN ASCOMYCETES-I

J. N. Kapoor and H. S. Gill

(Accepted for publication July 30, 1961)

In this series of papers, it is proposed to record ascomycetes hitherto not known from India. The present paper gives an account of 3 new species and 4 new records. Two new combinations are also proposed. Some of the specimens reported here were collected more than 45 years back and are still in remarkably good condition. The specimens have been deposited in Herb. Crypt. Ind. Orient., New Delhi and are indicated in the text by H.C.I.O. numbers.

1. *Anthostomella jasmini* Sp. Nov.

Stromata nigra, plus minusve rotundata atque tenuia; perithecia immersa in matricem, singula vel aggregata, nigra, globosa vel subglobosa, ostilo brevi vel nullo; perithecii parietes fusce brunnei vel nigri; asci semper cylindrici, octospori 77 – 121 x 10 – 13μ; ascosporae uniseriate, fusce brunneae vel nigrescenti-brunneae, naviculares, contiruae, 2 – 3 globulis oleaceis inclusis, 15 – 18 x 7 – 9μ; paprahyses filiformes, septatae, ascis multo largiores.

Typus lectus in *Jasmino auriculato* Vahl. e familia Oleacearum ad Pusa in prov. Bihar die 3 maii anni 1917 a M. Taslim, H.C.I.O No. 26841.

Stroma black almost round, thin; perithecia sunken in the matrix, single or aggregated, black, globose to sub-globose with an inconspicuous ostiole. Walls of the perithecia dark brown to black; asci cylindrical, 8-spored, 77– 121 x 10 – 13μ ; ascospores uniseriate, darkbrown boat-shaped, continuous with 2 – 3 big oil drops, 15 – 18 x 7 – 9μ; paraphyses filiform, septate, considerably bigger than the asci.

On *Jasminum auriculatum* Vahl. (Oleaceae), Pusa, Bihar, 3–5–1917 (M. Taslim), H.C.I.O. No. 26841. (Type).

2. *Asterinella loranthi* Sydow, Enum. Philipp. Fungi II in Philipp. Jour. of Science **8** : 490, 1913; Sacc. Syll. Fung. **24** : 482, 1926.

On living leaves of *Loranthus* sp. (Loranthaceae). Pusa, Bihar, 3–5–1917 (M. Taslim), H.C.I.O. No. 26843.

The fungus produces black irregular patches, upto 2 mm. in size, on the upper surface of the leaves. Free mycelium is creeping, dark brown and devoid of hyphopodia. Ascocarps are almost round, radiate, measuring 280–400 x 200–380μ in size. Asci are sub-globose to slightly oblong, 4–spored and 50–65 x 40–50μ in size. Ascospores are ovate-oblong, 2-celled

with a constriction at the septum, young spores at first hyaline turn olive brown with age, 32-40 x 18 – 22μ in size.

3. *Aulographium reticulatum* Phil. & Hark. in Grev. **14** : 23.

On leaves of *Quercus* sp. (Fagaceae), Catchment area, Simla, May, 1955 (J. N. Kapoor), H.C.I.O. No. 26186.

The ascocarps are scattered in patches and are superficial. They are spherical to elongated, convex, adnate, measure 250–350μ in diameter and open by longitudinal slit along the centre. Asci are at first sub-globose then becoming obovate and measure 16 – 24 x 12 – 14 μ. Ascospores are inordinate to biseriate, uniseptate, hyaline ovate-oblong and tapering below.

4. *Mycosphaerella aethiops* (Fuck.) Comb Nov. as *Sphaeralla aethiops* Fuck., in Symb. myc. p. 106, 1869.

On leaves of *Quercus* sp. (Fagaceae), Sikkim, 10–4–1957 (J. N. Kapoor), H.C.I.O. No. 26212. The ascocarps are small, black, thickly gregarious formed in angular spots on the under surface of the leaves, They are limited by veins, innate, punctiform, globose, and 55 –80μ in size. Asci are clavate-cylindric and measure 24 – 26 x 7 – 8μ Ascospores are biseriate, obovate-oblong, uniseptate with constriction at the septum They are hyaline and measure 6 – 8 x 2– 3 μ

5 *Mycosphaerella minimaepuncta* (Cks.) Comb. Nov. as *Sphaerella minimaepuncta* Cke., in Jour Bot , 1883.

On leaves of *Gladiolus* sp. (Iridaceae), Kalimpong, Bengal, April, 1957 (J. N. Kapoor), H.C.I.O. No. 26184.

The ascocraps are black, punctiform, erumpent and measure 70 – 90μ in diameter. Asci are clavate and short stipitate. Ascospores are single-celled, hyaline, narrow elliptical and measure 8–11 x 3.5μ in size.

6. *Otthia quercicola* Ell. & Ev. in N. Amer. Pyren. 250, 1892.

On dead leaves of *Quercus* sp. (Fagaceae), ) Sikkim, April 1957 (J. N. Kapoor), H.C.I.O. No. 26134.

The ascocarps are globose to subglobose, caespitose black, carbonous and measure 200 – 250μ in diameter. Asci are clavate, cylindrical, paraphysate and measure 60 – 110 x 16 – 18μ Ascospores are obliquely monostichous to crowded, ovate oblong, rounded at apex and tapering below They are light brown in colour, uniseptate, the lower cell narrower and measure 20 – 24 x 10 – 12μ

7 *Pringsheimia cynodontis* Sp. Nov.

Perithecia subglobosa, dispersa, innata, postea erumpentia, fusce brunnea, glabra, parietibus tenuibus et membranaceis, 50 – 80μ diam.; obclavata vel. obovata, apice crassiore, aparaphysata, octospora, 60 – 70


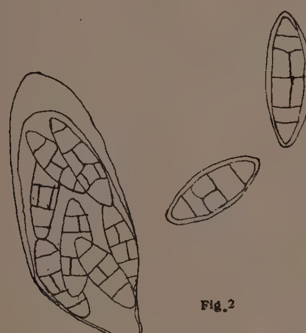




Fig. 1. *Anthostomella jasmini*

Fig. 2. *Pringsheimia cynodontis*

Fig. 3. *Valsaria bambusae*

x28 – 32μ; ascosporae irregulariter biseriatae vel aggregatae, cylindricae, fastigatae infra, apicibus rotundatis, 4-spetatae septis 2 – 3 longitudinalibus, saepe semel constrictae ad medium, magnit, 28 – 32 x 10 – 12μ.

In foliis marcescentibus *Cynodontis dactyli* e familia graminearum mense, januaris 1957, in regione. Instituti Mycologici I.A.R,I., ad New Delhi, J. N. Kapoor, H.C.I.O, No. 26868.

Perithecia sub-globose, scattered, innate, later erumpent, dark brown, glabrous, wall thin and membranous, 50 – 80μ in diameter; obclavate to obovate, thickened at the apex, aparaphysate, 8-spored, 60–70 x 28–32μ; ascospores irregularly biseriate or crowded, cylindric, tapering below, ends rounded, 4-septate with 2 – 3 longitudinal septa, often once constricted in the middle, measuring 28 – 32 x 10–12μ.

On drying leaves of *Cynodon dactylon* L. (gramineae), January, 1957, Mycological area, I.A.R.I., New Delhi J. N. Kapoor, H.C.I.O. No. 26869. (Type).

8. *Valsaria bambusae* Sp. Nov.

Stromata linearia nonnum que plurea simul , nigra, 2 – 5mm. longa; perithecia urceolata, ostiolo conico vel elongato, nigra; asci oblongi, pedicellati, octospori, uniseriati, paraphysibus, filiformibus, 119 – 144 x 14μ. ascosporae oblongae, paulum angustatae ad utrunque apicem, semel septatae, fumoso-brunneae, 20 – 22 x 10 – 11μ.

Typus lectus in culmis *Bambusae* sp. e familia graminearum ad Dharwar, in prov. Bombay, maio mense anni 1915 a G. S. Kulkarni, H.C.I,O. No 26844.

Stroma linear, at times many running together, black, 2 – 5 mm. long; perithecia flask-shaped, ostiole conical or elongated, black; asci oblong, stalked, 8-spored, uniseriate with filiform paraphyses 119 – 144 x 10 – 14μ; ascospores oblong, both ends slightly narrowed, 1-septate, smokybrown in colour, 20–22x 10 – 11μ.

On stems of *Bambusa* sp. (Gramineae), Dharwar, Bombay, May, 1915 (G. S. Kulkarni), H.C.I.O. No. 26844 (Type).

9. *Venturia circinans* (Fr.) Sacc. in Syll Fung. **1** : 529, 1882.

On leaves of *Geranium wallichianum* Sw. (Geranicaceae), Chakrata, (K. Bagchee), HC.I.O. No. 26842.

The ascocarps are formed on the surface of the leaves gregariously. They are sub-globose, measuring 65 – 131 μ in diameter and crowned above with 5 – 8 divergent broad curved, black bristles, 14 – 29 x 4μ in size. Asci are oblong cylindrical, sessile, aparaphysate, 8-spored and measure 40–61 x 7–11μ. Ascospores are hyaline, crowded or biseriate, uniseptate with upper cell broader, fusiform and measure 11 – 14 x 4 – 5 μ.

*Venturia glomerata* Cke. which is recorded in *Geranium dissecta* has much smaller and narrower ascospores.

Our grateful thanks are due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology, for his keen interest and helpful criticism. We also thankfully record the help of Rev. Father H. Santapau for translating the diagnosis of new species into latin.

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# FURTHER STUDIES ON THE SUSCEPTIBILITY OF SOME GRASSES TO CEREAL RUSTS

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Importance of collateral hosts in the perpetuation of different rusts of cereals has been well appreciated. Some wild grasses are known to harbour one or more rusts in nature while others are known to get infected with rusts under conditions of artificial infection. Attempts have been made in several countries to determine the nature of relationship between grasses and cereal rusts and the role of the former in perpetuation or dissemination of the latter.

In India natural occurrence of *Puccinia graminis* Pers. and *P. glumarum* (Schm.) Erikss. & Henn. has been recorded on some grasses by Butler (1918) and Butler and Bisby (1931). Presence of *Puccinia graminis tritici* (Pers.) Erikss. & Henn. has been recorded on a few grasses by Mehta (1940). Susceptibility of some indigenous and exotic grasses to black rust of wheat and oats and yellow rust of wheat was demonstrated by Prasada (1948) by artificial experiments. He also recorded occurrence of wheat black rust on 12 exotic grasses in nature (1951). Vasudeva, Joshi and Lele (1953) tested 29 grasses under artificial conditions with Indian physiologic races of black rusts of wheat and oats, brown rust of wheat, and yellow rust of wheat and barley and determined their susceptibility to each one of these rusts.

Experimental: Twelve races and bio-types of *Puccinia graminis tritici*, 9 races of *Puccinia triticina*, 10 races of *Puccinia glumarum* and 4 races of *Puccinia graminis avenae* from type culture collection were used in separate mixtures.* A total of 64 grasses were inoculated in different seasons and their reactions against the mixture of races of black, brown and yellow rust of wheat and black rust of oats were separately recorded. Results of those showing susceptibility are summarised in the Table. It is evident from the table that seedlings of only four genera (*Agropyron*, *Aegilops*, *Phalaris* and *Avena*) were found to get infected by the rusts under study. *Agropyron longiaristatum*, *A. semicostatum*, *Aegilops biuncialis*, *A. longissima*, *A. ventricosa*, *A. caudata*, *A. cylindrica*, and *A. sharonensis* were either moderately or heavily susceptible to *P. g. tritici*. With *Puccinia triticina*, *Agropyron semicostatum*, *Aegilops triuncialis* and *A. squarrosa* showed moderate infection. Tests with *P. gulmarum* showed that *Agropyron longiaristatum*, *A. semicostatum* and 10 species of *Aegilops* viz. *A. longissima*, *A. crassa*, *A. ovata*, *A. ventricosa*, *A. bicornis*, *A. columnaris*, *A. cylindrica*, *A. sharonensis*, *A. squarrosa* and *A. triaristata* showed moderate or heavy infection.

* Races 15, 15-C, 21, 24, 34, 40, 42, 42-B, 75, 117, 122 & 194; races 10, 11, 20, 26, 63, 77, 106, 107 & 108; races 13, 19, 20, 31, A, D, E, F, G & H and races 3, 4, 6 & 7 respectively.

Black rust of oats, *P. graminis avenae* infected very few grasses. *Avena glauca* was moderately infected, but *Phalaris canariensis*, *P. minor*, *P. tuberosa*, *Agropyron triticeum*, *A. longiaristatum* and *A. semicostatum* showed light infection. Rest of the grasses were immune to this rust.

All the 4 species of *Agropyron* and 14 of *Aegilops* showed. in field plots at the Wheat Breeding Sub-Station, Simla, traces to light infection of black rust and moderate to heavy infection of yellow rust under natural conditions.

Greenhouse tests on *Agropyron longiaristatum*, *A. semicostatum*, *Aegilops caudata*, *A. triuncialis*, *Phalaris minor*, *P. canariensis* and *P. tuberosa* with three rusts of wheat and black rust of oats have been reported earlier (Prasada, 1948, Vasudeva *et al*, 1953). During an epidemic in 1935 in N. Dakota in U.S.A, Young (1937) found *Agropyron semicostatum* to produce abundantly infected heads. Guyot (1946) from his experimental studies found the infection on *Aegilops ovata*, *A triaristata* and *A sharonensis* to be that of *P. g. tritici*. Marshall and Steyart (1930) observed *P. graminis* on *Panicum maximum*. In the course of present studies, the results of previous workers have been confirmed except that *Panicum maximum* showed immunity.

*Aegilops cylindrica* was reported by Melchers (1924) and Transchel (1934) to be susceptible to *P. triticina*. Johnston (1940) from his laboratory tests reported that whereas *A. ovata* L. is resistant to all the 5 physiologic races (viz. 5, 9, 15, 28 and 37) in Manhattan, U.S.A., *A. ovata* var. *globulosa* Zhuk. is susceptible to all the five races. He also recorded that *A. crassa* var. *refusans* Popov and *A. sharonensis* are susceptible to these races and *A. ventricosa*, *A. triaristata*, *A. cylindrica*, *A. triuncialis*, *A. biuncialis*, *A. columnaris*, *A. squarrosa*, *A. speltoides* and *A. caudata* are resistant. Guyot (1946) recorded the susceptibility of *A. squarrosa* and *A. triuncialis* to *P. triticina*. Critopoulos (1953) reported from Greece the susceptibility of *A. caudata* and *A. crassa*. In the present studies, however, all the species of *Aegilops* showed light to moderate type of infection.

Tschermak (1923) stated that in Austria, Hungary and Czechoslovakia *Aegilops ovata*, which is resistant to *P. glumarum* in spring, developed severe infection when sown in autumn. He summarised that precocity of development is undoubtedly the decisive factor in infection by yellow rust, inherent varietal susceptibility being secondary. Bremer (1947), from Turkey, recorded the susceptibility of *Aegilops cylindrica* and *A. ovata* to *P. glumarum*, and Petrak (1941) from Greece recorded its occurrence on *A. triuncialis*. Ling (1945) in China found that *P. glumarum* is commonly found on *Agropyron semicostatum* and *A. ciliare*. The findings of Tschermak, Bremer, Petrak and Ling have been confirmed during the present investigations.

Fischer and Levine (1941) reported that *Phalaris canarienis* is susceptible to *P. graminis avenae* under field and laboratory conditions; whereas Vallega (1943) from Argentina reported a certain degree of susceptibility of this grass to *P. g. avenae*. In the present studies, *P. canariensis* and *P. minor* showed light type infection and *Avena glauca* showed

Reaction of 22 grasses to black, brown, and yellow rusts of wheat and black rust of oats

| Name | *P. g. tritici* | | *P. triticina* | | *P. glumarum* | | *P. g. avenae* | |
|---|---|---|---|---|---|---|---|---|
| | No. of seedlings infected. | Reaction | No. of seedlings infected. | Reaction | No. of seedlings infected. | Reaction | No. of seedlings infected | Reaction |
| *Avena glauca* | 0/29 | 1 | 0/14 | I | 0/15 | I | 18/18 | S Moderate |
| *Phalaris canariensis.* | 0/20 | I | 0/20 | I | 8/8 | SR Light | 22/22 | S Light |
| *P. minor* | 0/21 | I | 0/20 | I | 0/14 | I | 20/20 | SR Light |
| *P. tuberosa* | 0/38 | R | 0/31 | R | 0/15 | R | 30/30 | SR Light |
| *Agropyron triticum* | 23/23 | S Light | 0/10 | R | 16/16 | S Light | 21/21 | S Light |
| *A. longiatistatum* | 22/22 | S Light | 18/18 | S Light | 22/22 | S Heavy | 22/22 | R |
| *A. semicostatum* | 28/28 | S Heavy | 19/19 | S Moderate | 22/22 | S Heavy | 15/15 | R |
| *Agropyron* sp. (Simla) | 12/12 | S Light | 1/8 | S Light | 8/8 | S Light | 0/8 | I |
| *Aegilops biuncialis* | 18/18 | S Moderate | 20/20 | SR Light | 12/12 | S Light | 0/14 | I |
| *A. longissima* | 20/20 | S Moderate | 22/22 | S Light | 20/20 | S Moderate | 0/8 | 1 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. crassa* | 19/19 | S | Light | 15/15 | S | Light | 18/18 | S | Moderate | 0/9 | I |
| *A. ovata* | 15/15 | S | Light | 20/20 | S | Light | 18/18 | S | Heavy | 0/12 | I |
| *A. triuncialis* | 12/12 | SR | Light | 16/16 | S | Moderate | 24/24 | SR | Light | 0/15 | I |
| *A. ventricosa* | 15/15 | S | Moderate | 12/12 | S | Light | 25/25 | S | Heavy | 0/12 | I |
| *A. bicornis* | 28/28 | S | Light | 14/14 | S | Light | 17/17 | S | Moderate | 0/9 | I |
| *A. caudata* | 22/22 | S | Moderate | 12/12 | SR | Light | 15/15 | SR | Light | 0/10 | I |
| *A. columnaris* | 19/19 | SR | Light | 15/15 | S | Light | 18/18 | SR | Heavy | 0/12 | I |
| *A. cylindrica* | 24/24 | S | Moderate | 17/17 | SR | Light | 20/20 | S | Heavy | 0/15 | I |
| *A. sharonensis* | 22/22 | S | Moderate | 16/16 | S | Light | 25/25 | S | Heavy | 0/12 | I |
| *A. speltoides* | 18/18 | R | | 12/12 | R | | 12/12 | SR | Light | 0/15 | I |
| *A. squarrosa* | 12/12 | S | Light | 10/10 | S | Moderate | 25/25 | S | Heavy | 0/12 | I |
| *A. triaristata* | 15/15 | S | Light | 20/20 | SR | Light | 22/22 | S | Moderate | 0/14 | I |

I = Immune

R = Flecking or drying of leaf tips. Pustules usually absent; if present minute and surrounded by necrotic zones.

S = Pustules large, without necrotic zones.

SR.= Both types of pustules on the same culture.

moderate infection. In Australia, Waterhouse (1929) recorded the susceptibility of *Phalaris minor* to this rust.

The following forty-two species of grasses (most of them fodder grasses) were also tested with mixture of all the races of three rusts of wheat and black rust of oats separately. *Cenchrus setigerus, C. ciliaris, Penniseum polatachyum, P. orientale, P. pedicillatum, Bothriochloa pertusa, B. inscupta, Iselema laxum, Dicanthium annulatum, Schima nervosum, Panicum antidotale, P. maximum, P. coloratum, Heteropogon contortus, Chloris bourni, Urochloa* sp., *U. mosambecensis, Eragrostis curvula, E. trichoides, E. chloromelas, E. lehmaniana, Mellinis minutifolia, Bracharia brizantha, Setaria sphacelata, S. glauca, Tricholaena rosea, Paspalum dilatatum, P. sanguinale, P. notatum, Digitaria* sp., *Sporobolus fimbriatus, S. wrightii, S. aeriodes, Paspalum sanguinale, Echinochloa colona, Oplismenus burmanii, Saccharum spontaneum,, S. munja, Erianthus hookeri, E. ravennae, Vetiveria zizanoides* and *Rottboelia* sp., None of the seedlings of these grasses took up infection.

Thus, it is found that some of the exotic grasses get infected under artificial conditions to one or more rusts occurring on cereals. The hazards involved in the indiscriminate introduction of grasses before testing them for susceptibility to cereal rusts, can hardly be ignored as they may, if given suitable conditions, play a significant part in perpetuation or dissemination of rusts from year to year in this country as well.

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*Originals not seen

# GLOMERELLA CINGULATA (STONEM.) S. & VON S. ON SUGARCANE IN INDIA

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(Accepted for publication July 15, 1961)

Red Rot of sugarcane, caused by *Colletotrichum falcatum* Went (= *Glomerella tucumanensis*), is the most serious disease of this important commercial crop in Northern India, and, off and on, assumes epiphytotic proportion, which has been ascribed to the development of more virulent strains of the causal organism (Chona, 1943). In connection with the study of occurrence of strains of *C. falcatum*, a very large number of isolations are being made from Red Rot affected cane-stalks of different varieties obtained from different areas in the country, as also cane leaves, where the fungus produces mid-rib lesions. During these studies, an isolate, very different from the usual ones, was obtained from the leaves of a collection made in January, 1960 at Samalkot (Andhra Pradesh) from cane variety Co. 527, which proved to be an altogether different fungus and has been identified as *Glomerella cingulata*. Since this fungus has not been recorded as pathogenic on sugarcane so far, some preliminary observations in this respect are reported here.

Symptoms: The disease is characterised by the production of leaf mid-rib lesions (Fig. 1), as blood-red elliptic spots, 4–10 mm. long and 4–6 mm. wide, which later enlarge and become dark brown, with straw coloured or light brown centres. In humid weather, numerous black dot-like acervuli are formed in the central discoloured portion, which contain numerous spores. The spots are isolated, but sometimes may coalesce to form bigger lesions. Except for their slightly darker colour, the spots are indistinguishable from those formed by *C. falcatum*, the Red Rot organism. No infection was observed on leaf-lamina or leaf-sheath.

On the withered cane stalks, black dot-like acervuli appear on the nodes or the growth-cracks in the internodes from which shiny, pinkish masses of spores ooze out. Under favourable conditions, numerous black, round perithecia appear around the acervuli. On splitting open the infected canes, the fungus is found in red streaks which follow the course of vascular bundles and run from one internode to another (Fig. 2), there being little lateral spread of infection in the cane-stalk. Typical alcoholic smell or white patches characteristic of Red Rot affected canes were absent in this case.

Morphology: In young lesions, the mycelium is subcuticular and occurs as thin, hyaline, interlaced but distinct hyphae. Later, the mycelium invades the cells of epidermis and becomes considerably gelatinised, making it difficult to differentiate the individual hyphae. The hyphae are closely septate and the cells are mostly 14–18$\mu$ in length and 5–6$\mu$ broad. The mycelium aggregates below the cuticle to form a linear stroma.

Fig. 1. Fig. 2.


Fig. 1. Sugarcane leaves showing leaf mid-rib lesions due to *Glomerella cingulata*.

Fig. 2. Diseased stalks of sugarcane.

1. Showing symptoms of red rot due to *C. falcatum*.
2. Showing streaks due to *G. cingulata*.
3. Mixed symptoms due to both the organisms.

The conidiophores arise as short, cylindrical, single-celled structures from the cells of the stroma and form a compact layer. The conidiophore tapers towards the apex and bears a single conidium on its tip. The conidia (Fig. 3) are hyaline, single-celled, oblong-elliptic, with both ends rounded, sometimes one end tapering; straight or slightly curved, with a thin epispore and granular contents, measuring 16–20 x 4–5μ and are pink *enmasse*. Setae are dark brown, straight or slightly curved, thicker below and pointed above (base 5μ and tip 2μ), generally 2-septate, somewhat constricted at the upper septum and measure 60–90μ in length, are rarely observed in the acervuli and are few in number.

The perithecia (Fig. 4) are globose, ostiolate, immersed totally in host tissues with only the beak protruding as short papilla and measure 130–390μ (average 260μ) in length (without papilla) and 104–250μ in breadth. The outer surface is parenchymatic and dark brown while the inner layers are hyaline. Numerous asci are borne within each perithecium. The asci

(Fig. 5) are hyaline, clavate to subfusiform, short-stalked or sessile, aparaphysate with a thick wall and an ostiole at the broad apex. These measures 68 - 75 x 10 - 12μ. The ascospores are arranged biseriately within the ascus, and are 8 in number. These are straight or slightly curved, somewhat fusoid, with both ends rounded, hyaline, single-celled and measure 15 – 18 x 3.5 - 4μ (Fig. 6).

Fig. 3. Fig. 4.

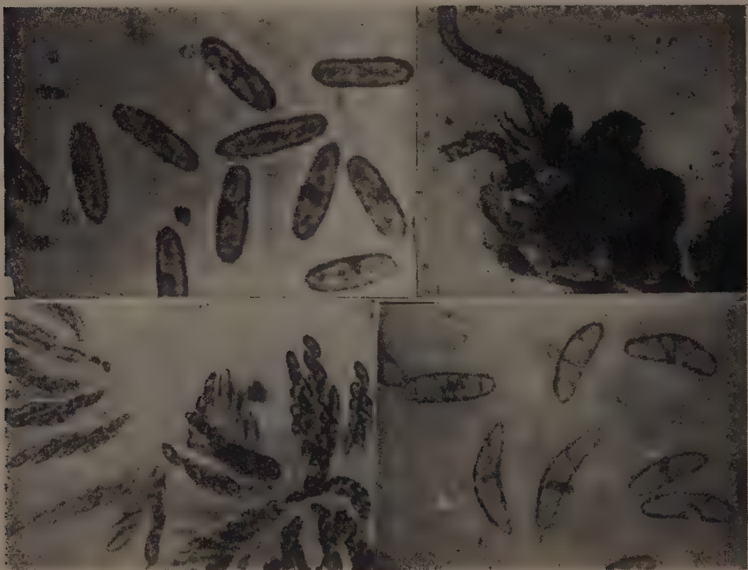


Fig. 5. Fig. 6.

Fig. 3. Conidia of *G. cingulata*.

Fig. 4. Perithecia of *G. cingulata* showing long beaks (in culture).

Fig. 5. A group of asci of *G. cingulata* with ascospores arranged biseriately.

Fig. 6. Ascospores of *G. cingulata*.

PATHOGENICITY: The pathogenicity of the fungus has been tested on cut canes, standing canes and the cane leaves. The cane stalks (Co. 312) were inoculated with the conidial suspension prepared in sterile distilled water, by the plug method. The cut-ends as well as the points of inoculation were sealed with melted wax to avoid driage, and the inoculated canes were wrapped in wet gunny-bags and stored at room temperature (22-27°C) and kept moist by sprinkling water once or twice a day as necessary. The controls were similarly treated except that only sterile distilled water was used as inoculum. After 3 weeks of incubation, the inoculated canes were cut open longitudinally and observed for the progress of infection. The inoculated ones were found to show a few typical red streaks running

through a few internodes, beyond the inoculated internode, while the controls remained unaffected.

In the case of leaf inoculations, the conidial suspension was sprayed on leaves of growing plants which had been slightly injured by fine needle punctures and kept in humid chamber at 23–26 °C for 48 hours and then placed in the open. Typical dark-brown spots appeared on mid-rib portion only. No sporulation, however, took place, presumably due to lack of proper humidity during the post-infection period. The lesions on the leaf mid-rib produced by *G. cingulata* are likely to be mistaken for those of Red Rot fungus under field conditions because of their great resemblance.

In order to study the associated effect, if any, of this fungus with the Red Rot organism, on sugarcane, standing canes of Co. 312 were inoculated by the standard plug method with both the organisms, individually as well as in mixture. The linear spread of infection was measured by splitting open, longitudinally, the inoculated canes after three weeks of making the inoculation and the results obtained are given below:

| *Pathogen* | | *Linear spread of infection* (in cm.) |
|---|---|---|
| *Glomerella cingulata* isolate | ... | 48.3 |
| *C. falcatum* (isolate 244) | ... | 48.8 |
| Mixture of *G. cingulata* and *C. falcatum* | | 52.3 |

It will be observed that there is hardly any difference in linear spread of infection in the case of the two fungi. The symptoms produced by the two organisms individually are, however, easily distinguishable, there being only a few red streaks in the case of *G. cingulata*, without any lateral spread in the cane stalk (Fig. 2).

In all cases, the fungi inoculated were successfully reisolated from the inoculated canes. Isolations made from the canes inoculated with a mixture of the two fungi, including those made from the junction of healthy and diseased portions, yielded both the organisms.

Using the method described by Carvajal and Edgerton (1944), old dry leaves of sugarcane (Co. 312) were inoculated with the conidial suspension of *G. cingulata*. Profuse perithecial production was observed all over the leaf surface (Fig. 7) within six days.

Cultural Studies: On oatmeal agar, the growth of the fungus was observed 24 hours after seeding at the room temperature (17–20 °C), which progressed rapidly, producing silky-white mycelium without imparting any change of colour to the substrate. The conidia appeared as pink spore masses on the third day and their intensity increased on further incubation of the cultures. The pink spore masses were soon replaced by grey to smoky-grey coloured mycelium in which black-dot-like perithecia were produced in abundance. Mature perithecia with asci and ascospores were observed on the sixth day in the culture. The intensity

of perithecial formation increased to such an extent that it completely superimposed the highly sporulating conidial culture (Fig. 8).

Fig. 7.

Fig. 8.

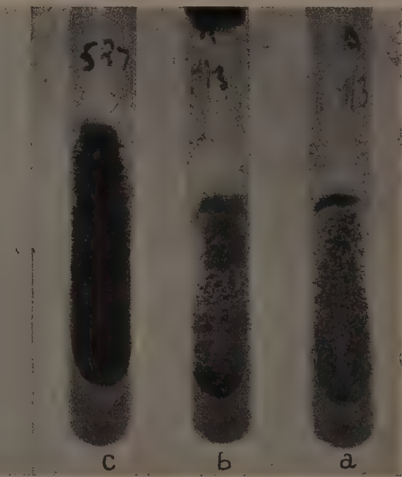



Fig. 7. Old dry (autoclaved) leaf of sugarcane showing profuse perithecial production, after inoculation.

Fig. 8. Culture of *G. cingulata* showing development of perithecia. (a) Profuse conidial production (b) Conidial masses partly superimposed by perithecia (bottom end) (c) Conidial masses completely superimposed by perithecia.

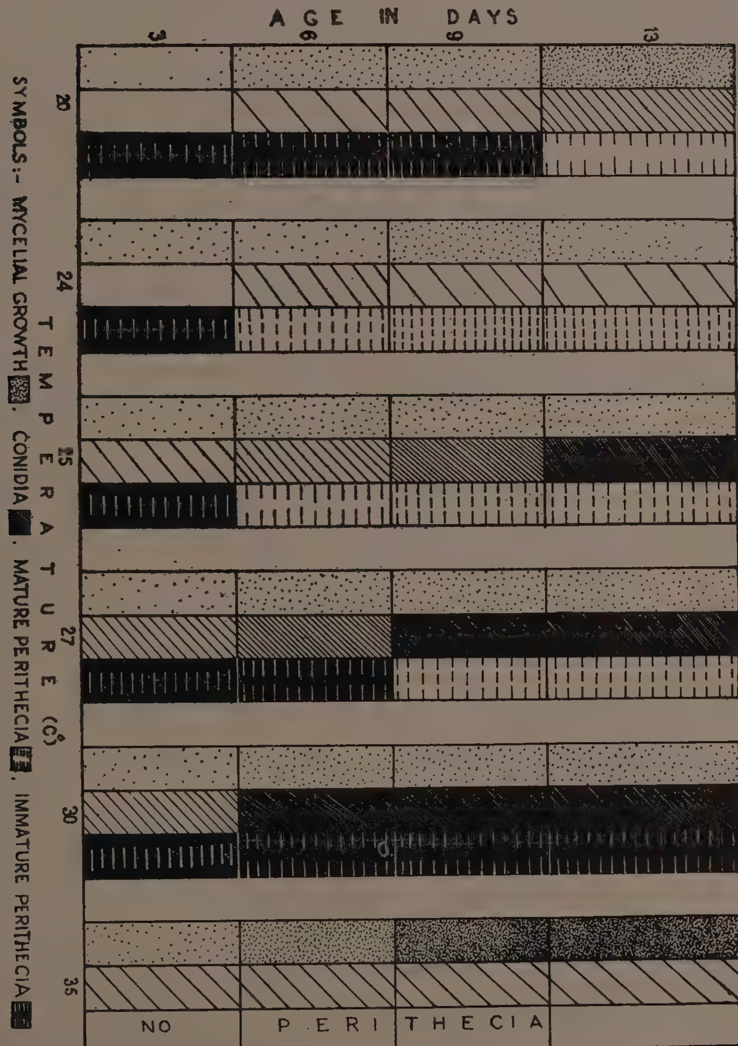
To study the effect of temperature on the development of perithecia and conidia, the cultures on oat-meal agar were incubated at different temperatures and observations recorded after 3, 6, 9 and 12 days. The data are presented in Text Fig. (i), which show that optimum temperature for the development of perithecia is 24 C and of conidia 30°C. The mycelial growth was more profuse at higher temperature (35°C), whereas the conidial sporulation was depressed appreciably and the perithecial formation inhibited. There was no growth at 10, 15 or 40°C.

In another experiment, the development of perithecia was studied on different media, e.g., Oat meal agar*, Potato dextrose agar** and Dextrose-asparagine-phosphate agar†, at 5 different temperatures (10, 15 27, 30 and 35°C). It was observed that mature perithecia developed only on oat-meal agar at 27 C after 6 days growth whereas the other two media

* Quaker oats 40 gm.; agar agar 20 gm.; Dist. Water 1000 c.c.

** Peeled Potato 200 gm.; Dextrose 20 gm.; Dist. water 1000 c.c. agar agar 20 gm.

† Dextrose 30 gm.; Asparagine 1 gm.; Potassium dihydrogen phosphate 1.5 gm.; magnesium sulphate 0.5 gm.; Dist. water 1000 c.c.; agar agar 20 gm.




Text Fig. (i) Effect of temperature on growth, conidial and perithecial formation of *G. cingulata* on oatmeal agar after 3, 6, 9 and 13 days.

The depth of shading in each symbol represents the relative intensity in each case.

did not support any perithecial production, though profuse conidial formation was observed at 27 °C.

The conidia as well as ascospores were found to germinate readily at room temperature (25–27 °C) in distilled water and formed abundant appressoria.

Single-ascospore and single-conidial cultures on oatmeal agar produced identical growth resulting in conidial and perithecial formation, indicating thereby that the fungus is homothallic.

It was observed that after the conidial formation in culture, the conidia germinated freely and that the germ tubes of two conidia fused to produce a broader mycelium which gave rise to perithecial formation; or the two conidia joined each other through a connection tube and the resulting mycelium, possibly after dikaryotisation, gave rise to perithecia (Fig. 9).

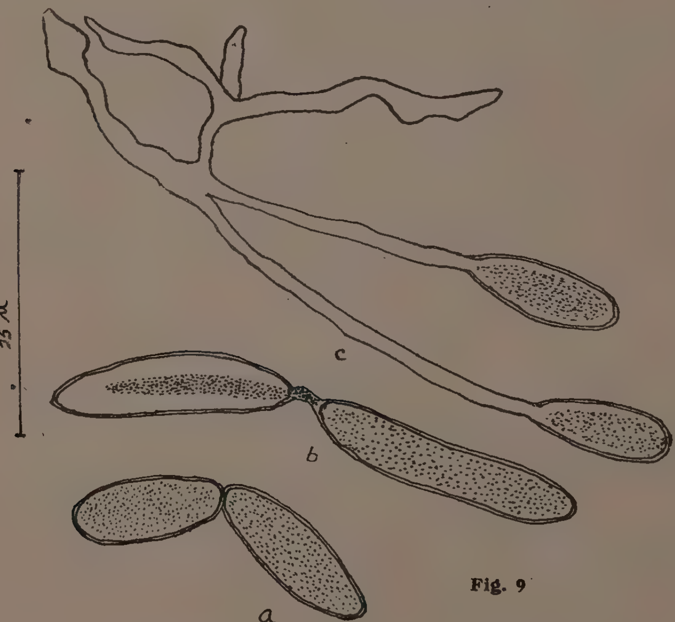



**Fig. 9**

Fig. 9. (a) Two conidia of *G. cingulata* showing conjugation.

(b) Contents of one of the conjugating conidium passing into the other.

(c) Fusion of germ-tubes of two conidia resulting into stouter common mycelium.

IDENTITY OF THE PATHOGEN: The conidial stage of the fungus agrees with the description of *Colletotrichum gloeosporioides* Penzig, as the mode of spore production, spore and conidiophore-characters, size and shape of conidia, etc. and the acervuli formation are typical of *C. gloeosporioides*. Furthermore, the perfect stage of *C. gloeosporioides* has been reported by Spaulding and von Schrenk as also by several other workers as *Glomerella cingulata*, with which the characters of the fungus described above compare favourably. The abundant perithecial formation is, however, typical of this isolate. The fungus under study is, therefore, identified as *Glomerella cingulata*.

Two species of *Colletotrichum* viz. *C. graminicolum* (Ces.) Wilson = *C. lineola* Corda, and *C. falcatum* Went = *Glomerella tucumanensis* (Speg.) Mueller and von Arx, have been recorded on sugarcane but both of these have falcate spores with more or less pointed ends and are, therefore, distinct from the fungus reported here which has elliptic spores with rounded ends.

## SUMMARY

A disease of sugarcane, affecting Co. 527 leaves, with symptoms closely resembling *C. falcatum* mid-rib lesions, but caused by *Glomerella cingulata* which is reported for the first time as pathogenic on sugarcane, has been described

In addition to the midrib lesions on cane leaves, the fungus is capable of parasitising cane-stalk where it produces only a few red streaks following the course of vascular bundles.

The fungus produces mature perithecia readily on oatmeal agar as also on the host in nature. The optimum temperature for perithecial production has been found to be 24 C, and for the conidial production 30°C. The fungus has been shown to be homothallic.

The conidial stage of the fungus closely resembles that of *Colletotrichum gloeosporioides* Penzig in all important aspects; and the perithecial stage with that of *Glomerella cingulata*. The abundant perithecial production, however, is characteristic of this isolate, which completely superimposes conidial production in 6 days old cultures. The *Colletotrichum* sp. commonly occurring on sugarcane, i.e. *C. falcatum*, has falcate conidia with more or less pointed ends and is thus distinct from the fungus reported here which has elliptic conidia with rounded ends.

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## BROAD BEAN MOSAIC VIRUS IN INDIA

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Introduction. During surveys of Kulu Valley (Punjab) and Himachal Pradesh a severe mosaic of Broad bean (*Vicia faba* Linn.) and Red clover (*Trifolium pratense* Linn.) was observed during 1957–1959. Investigations were made, with regards to the host-range, symptomatology, modes of transmission, and the properties of the causal virus which is hereafter referred to as BBMV.

Materials and Methods: The culture of the virus was maintained throughout the course of investigations on *Vicia faba* Linn. Beck's Green Gem and *Trifolium pratense* Linn. plants in an insect-proof house and the routine precautions were taken to prevent insect build-up and other ways of contamination.

Sap inoculations were carried out with crude juice from infected Braod bean leaves. The leaves of the plants intended for inoculation were first dusted on the upper surface with carborundum powder and then rubbed with cotton pads saturated with the infectious juice. After inoculation the leaves were washed with the tap water and the plants were kept in the glass house for observation.

The aphids used in insect transmission studies were raised on their respective healthy host plants. Prior to an access of about 15 minutes on diseased leaves the aphids were starved for nearly 2 hours and then they were left on the test plants (8–12 days old Beck's Green Gem Broad bean) for 2–4 hours. The test plants were then sprayed with nicotine sulphate to kill the aphids and maintained in a glass house.

Symptoms of the Disease: The affected Broad bean and clover plants first showed clearing of veins, and later severe mosaic mottling, slight puckering and smalling of the young leaves and stipules (Fig. 1). Diseased plants were stunted and produced fewer pods. No symptoms on pods were, however, observed.

Experimentation: Transmission; The virus was readily transmitted by juice inoculation, as well as, by aphids viz., *Aphis craccivora* Koch, *Aphis rumicis* Linn., *Macrosphoniella sanbornii* Gill., and *Myzus persicae* Sulz. The relationship between above aphid vectors and the BBMV appears to be of the non-persistent type as the viruliferous aphids do not retain the virus for more than two hours.

Host-range and Symptomatology; The virus was transmitted by juice inoculation, to *Vicia faba* Linn. vars. Beck's Green Gem, Broad Taylor's and Sutton's Early; *V. sativa* Linn.; *V. villosa* Roth.; *V. tetrasperma*

Linn.; *Crotalaria juncea* Linn.; *Glycine max* Merr.*; *Lupinus leuteus* Linn.; *L. albus* Linn.; *L. annus* Linn.; *L. texanus* Linn.; *Lathyrus odoratus* Linn. vars. Vulcan. Monarch, and Josie; *L. sativus* Linn.; *Medicago hispida* Linn.; *Melilotus alba* Lam.; *Trigonella foenumgraecum* Linn.; *Trifolium incarnatum* Linn.; *T. repens* Linn.; *T. pratense* Linn.; *Pisum sativum* Linn. vars. American Wonder, Alderman, and Alaska. The symptoms produced on some of the above mentioned host plants are described below;—

*V. faba var. Beck's Green Gem:* About a week after inoculation, intense vein clearing of the new leaflets developed, which was followed by severe mosaic mottling and slight puckering (Fig. 1). The symptoms were mostly confined to the younger leaflets. The infected plants were somewhat stunted and produced only few pods.

*Crotalaria juncea:* After about 20 days of inoculation the young leaves begin to show vein clearing which was eventually replaced by severe mosaic mottling and vein banding (Fig. 2). Old leaves did not show any type of symptoms.

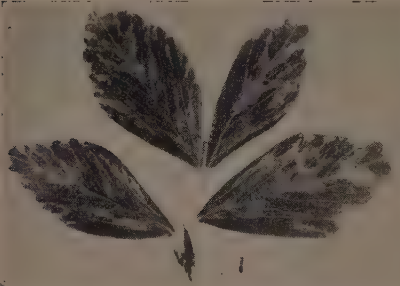

Fig. 1. Mosaic and slight rolling on *Vicia faba* var. Beck's Green Gem.

Fig, 2. Mosaic and vien banding on *Crotalarta juncea.*

*Lathyrus odoratus var. Josie:* The reaction of variety Josie was usually more severe than that of the variety Monarch. The new leaves developed a severe blotchy light green mottle accompanied by vein banding after about 2–3 weeks of inoculation. The leaves were greatly reduced in size. The mottling was observed on the stipules. The petals showed mild breaking.

*Lupinus albus:* About 2 – 3 weeks after inoculation, the young apical leaves showed necrotic streaks and spots and gradually the leaves withered away. Subsequently, necrotic streaks appared on the petioles, as well as, on the stem and the entire plant, which remained stunted, gradually died. In certain cases the inoculated leaves developed mild mottle and vein banding.

* Only 2 plants out of about 20 inoculated, had developed mosaic, from which the virus was also recovered.

*Melitotus alba*: First indication of the infection was the appearance of mild vein clearing on the new leaflets. Later the diffused pale yellow mottling developed which was sometimes followed by production of necrotic specks along the periphery. Frequently ring-spots developed on the leaflets.

*Pisum sativum var. Alaska*: About 3 – 4 weeks after inoculation, the young apical leaves showed a conspicuous pale mottle with vein banding (Fig. 3). Mild symptoms were also visible on the stipules. The leaves were very much reduced in size. Generally no mottling was observed on the pods.

*Trifolium incarnatum*: After about 20 days of inoculation the new leaves showed a severe pale blotchy mottle and vein banding (Fig. 4). Occasionally bronze streaks developed on the mid-rib which was more conspicuous on the dorsal side. The leaves were slightly reduced in size.

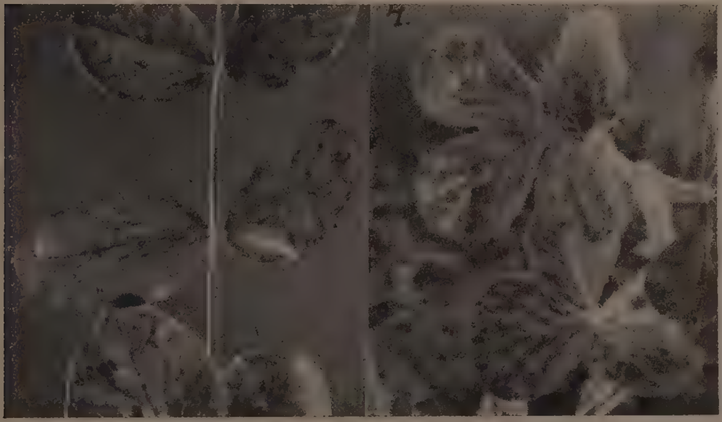

Fig. 3. Mosaic on *Pisum sativum* var. Alaska. Fig. 4. Mosaic and vein banding on *Trifolium incarnatum*.

*Trigonella foenum-graecum*: About two weeks after inoculation, intense vein clearing developed on the young leaves, which was followed by mild mottling. The entire plant remained stunted and gradually died.

The following plants species were resistant to the virus; *Arachis hypogea* L.; *Cajanus cajan* L.; *Cicer arietinum* L.; *Dolichos biflorus* L.; *D. lablab* L.; *Lupinus angustifolius* L.; *Medicago sativa* L.; *Pisum sativum* L. vars. Blue Bantom, Excelsior, Little Marvel, and Perfection; *Phaseolus vulgaris* L. vars. Beauty, Bountiful, Canadian Wonder, Dwarf, Earliest of White, Idaho, Refugee, Kentucky Wonder, Red Valentine, Tender Green, and Tender Yellow; *P. aureus* Roxb.; *P. lunatus* L.; *P. mungo* L.; *Brassica*

spp.; *Capsicum frutescens* L.; *Cucumis sativus* L.; *Datura* spp.; *Gomphrena globosa* L ; *Hesperis matronalis* L ; *Lycopersicon esculentum* Mill.; *Nicotiana tabacum* L.; *N. glutinosa* L.; *Solanum nigrum* L.; *Zinnia elegans* L.; and *Gladiolus* sp.

*Properties of the virus*· All tests were carried out on young plants of *Vicia faba* var. Beck's Green Gem which was considered to be the most suitable host plant.

Various dilutions of the leaf-extracts from diseased plants, made in distilled water, were found to be infective in dilutions from 1 x $10^{-2}$ to $10^{-3}$ but not in dilutions beyond 1 x $10^{-3}$. The virus in the undiluted leaf extracts was found to withstand exposure to 50-55 °C. but not to the temperatures exceeding 55°C. The samples of the virus, in crude juice, retained at room temperature (10–15 °C.) were infectious after 72 to 120 hours storage but not after 144 hours storage.

The leaf-extracts were diluted 10 times in 0.1 M-dipotassium phosphate buffer solutions adjusted to different pH values. Inoculations were immediately made after dilutions and it was found that the optimum range of H—ion concentration for infection was from pH 8 to pH 10.

The virus was recovered from the living roots (tap, as well as secondary and tertiary), stems, stipules, leaves, whole flowers, calyx, corolla, androecium and gynaecium but not from root nodules, pollen grains, and dried parts of the Broad bean plants.

The seeds obtained from the infected Broad bean plants of the variety Beck's Green Gem, *Vicia villosa*, Sweet pea variety Josie, and Garden pea variety Alderman were sown and the seedlings were examined. Among approximately 50–550 seedlings of each type no diseased plants developed.

DISCUSSION; Although a number of plant viruses have been reported to be naturally occurring on Broad Bean (Weiss, 1939; Smith, 1957), only those resembling the BBMV call for comparison. Boning (1927) described a mosaic disease of Broad beans transmissible to *Trifolium pratense* and *T. incarnatum*. The causal virus is not seed transmitted but is transmitted by certain aphids, cicadas and thrips. No information is, however, available on the physical properties of the virus. Pea mosaic virus has been reported to infect Broad bean by Doolittle and Jones (1925) Zaumeyer and Wade (1936), Chamberlain (1936), Murphy and Pierce (1937) and Chaudhary (1950). The causal virus (*Pisum virus 2* or *Marmor leguminosarum*) is transmitted by the aphids, *Macrosiphum gei*, *Myzus persicae* and *Aphis rumicis* but not through seed (Smith, 1957). *Phaseolus vulgaris* and American pea varieties Perfection and Horal are resistant to the virus. Stubbs (1937) reported three strains of the virus differentiated from each other in symptomatology and characterised by their inability to infect *Trifolium pratense*. The BBMV reported herein appears to resemble closely pea mosaic virus in physical properties, host range and aphid transmission and obviously belongs to the *Pisum* virus 2 or *Marmor leguminosarum* group. However, because of the ability of BBMV to infect soyabean, the virus is designated *Pisum* virus 2D or *Marmor leguminosarum* var. *varians*.

SUMMARY

A mosaic disease of Broad bean and Red clover occurring in Punjab and Himachal Pradesh has been reported.

The causal virus is sap-transmissible but is not transmitted through seed. *Aphis craccivora*, *A. rumicis*, *Macrosiphoniella sanbornii*, and *Myzus persicae* proved to be the vectors of the virus. The virus is of non-persistent type and the host range is limited to the family Leguminosae.

The virus has a thermal inactivation point of 55–60°C, a dilution-end-point between 1 x 10–3 and 1 x 10–4, longevity *in vitro* of 120–144 hours and optimum pH range for infection from pH 8 to 10. The virus has been shown to belong to *Pisum* virus 2 groups.

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* Original not seen.

## STUDIES ON POWDERY MILDEWS FROM INDIA-II*

H. S. Gill, R. L. Munjal and B. L. Chona

(Accepted for publication July 30, 1961)

The present paper deals with seven more species of Powdery Mildews collected from Kashmir, Dalhousie and Kulu Hills in the Himalayas, one of which is new, five are new records and one new host record for India. The specimens have been deposited in Herb. Crypt. Ind. Orient., I.A.R.I., New Delhi and their accession numbers are indicated in the text.

*Erysiphe communis* (Wallr.) Link. in Sp. Plant. **6** : 105, 1824; Blumer, Beit. zur Krypt. flora der Schw. Band 7, Heft 1, p. 177, 1933.

Amphigenous; mycelium persistent, thin, effused, conidia solitary rarely in chains, oblong or ellipsoidal, 29 – 36 x 14 – 18μ; perithecia scattered to gregarious, 90 – 125μ in diameter; cells of the wall 8– 15 μ wide; appendages variable in number and length, basal, hyaline, mycelial, numerous, 1 – 3 times the diameter of the perithecia; asci innumerable, ovate to broadly ovate, with or without a short stalk, measuring 54 – 65 x 29 – 36μ; with 3– 6 ascospores, 18 – 24 x 10 – 14 μ.

On living leaves of *Desmodium* sp. (Leguminosae), H.C.I.O. No. 27034, Dalhousie, Punjab, 9–11–58 (H. S. Gill).

*Erysiphe convolvuli* DC. in Flora Francaise **2** : 274, 1805; Blumer, Beit. zur Krypt. fl. der Schw. Band **7** Heft 1, p. 205, 1933.

Amphigenous; mycelium well-developed; conidia oblong, 36 – 40 x 11 – 16μ; perithecia scattered to gregarious, 90 – 126 μ in diameter, cells upto 18μ wide, appendages basal, numerous, 1½ to 2 times the diameter of the perithecia, subhyaline to light brown sometimes branched, asci numerous, 50–72 x 29–43μ; Asco-spores 3–6 measuring 22–25 x 10–15μ.

On living leaves of *Convolvulus arvensis* L. (Convolvulaceae), H.C.I.O. No. 27035, Shadipura (Kashmir), November, 1955 (R. L. Munjal).

*Phyllactinia andrachnes* sp. nov.

Hypophylla; mycelium evanescent; perithecia dispersa, ampla, globosa vel subglobosa, 180 – 216μ diam., cellulis usque ad 18.0μ latis, appendices 6 – 11 numero, 1 – 1½ latiores perithecia, rigidae, aciculares, rectae, aseptatae, incolorae, tumescentes ad basim in bulbum vacuum. Asci plurimi, subcylindrici vel ovato-oblongi, 65 – 72 x 29 – 32μ, pedicellati, bispori; sporae 25 – 36 x 15 – 22μ, oblongae vel irregulares forma et largiores ad unum apicem.

*No. I of the series appeared in Indian Phytopath. Vol. XIII. No. 1. pp. 71-75, 1960.

In foliis viventibus *Andrachnes cordifoliae* Muell. (Euphorbiaceae) H.C.I.O. No. 270,36 ad Kulu (Punjab), 17 novembris anni 1959 (H. S. Gill and Gian Singh).

Hypophyllous; mycelium evanescent, perithecia scattered, large, globose to sub-globose, 180 – 216$\mu$ in diameter; cells upto 18.0$\mu$ wide; appendages 6 – 11 in number, 1–1$\frac{1}{2}$ times the diameter of the perithecia, rigid, acicular, straight, aseptate, colourless, swollen at the base into a hollow bulb; asci numerous, sub-cylindrical to ovate-oblong, 65 – 72 x 29 – 32 $\mu$, stalked, containing 2 ascospores, 25 –36 x 15 – 22$\mu$, oblong, sometimes larger at one end.

On living leaves of *Andrachne cordifolia* Muell. (Euphorbiaceae), H.C.I.O. No. 27036, Kulu, Punjab, 17–11–1959 (H. S. Gill and Gian Singh).

*Phyllactinia corylea* (Pers.) Karst. in Act. Soc. Faum. Fl. Fenn. 2 : 95, 1885, Salmon, Monograph Erysiphe, p. 224, 1900.

Hypophyllous; mycelium more or less persistent; conidia solitary; granulate, clavate, 43 – 58 x 18 – 22 $\mu$; perithecia scattered, globose to subglobose, 184 – 236 $\mu$ in diameter; cells 14 – 22 $\mu$ wide; appendages 5 – 13, 1 – 2 times the diameter of the perithecium, acicular, rigid, straight, aseptate, colourless, swollen at the base into a hollow bulb; asci numerous, subcylindrical, 61 – 79 x 29 – 36 $\mu$ more or less stalked, Asco-spores 2, rarely 3, oblong, sometimes curved, 25 – 32 x 14 – 18$\mu$.

On living leaves of *Desmodium* sp. (Leguminosae) H.C.I.O. No. 27037; Dalhousie, Punjab, 9–11–1953 (H. S. Gill).

Fig. 1.

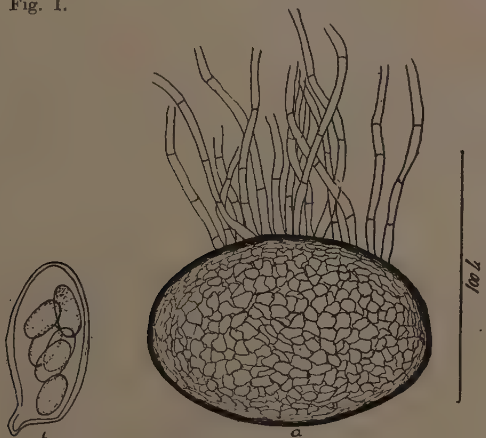



Fig. 1. *Erysiphe communis*

(a) Perithecium

(b) Ascus and Ascospores

Fig. 2.

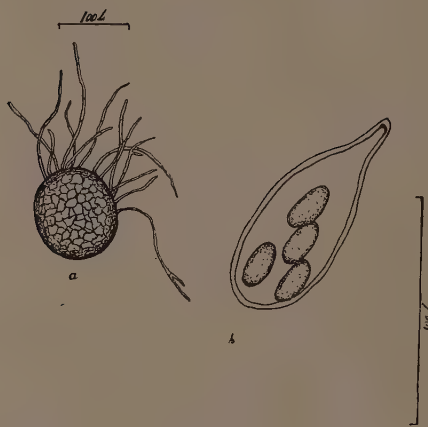


Fig. 2. *Erysiphe convolvuli*
(a) Perithecium
(b) Asci and Ascospores

Fig. 3.

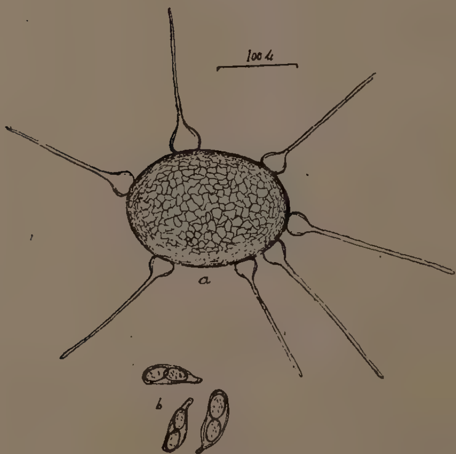
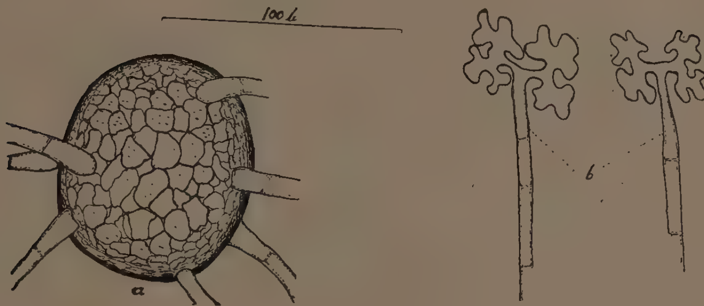


Fig. 3. *Phyllactinia andrachnes*
(a) Perithecium
(b) Asci and Ascospores

Fig. 4. *Uncinula clintonii*
(a) Appendages
(b) Asci and Ascospores

Fig. 4.

Fig. 5.



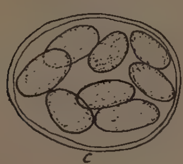
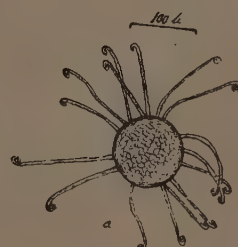


Fig. 5. *Podosphaera oxyacanthae*
(a) Perithecium
(b) Appendages
(c) Ascus and Ascospores



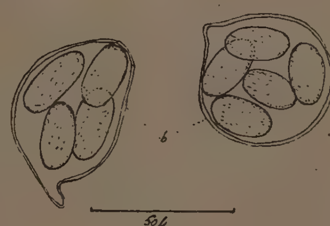


Fig. 6. *Uncinula mori*
(a) Perithecium
(b) Asci and Ascospores

Fig. 6.

*Uncinula clintonii* Peck. in Trans. Albany Inst. 7 : 216, 1872, Homma, Jour. Hokk. Imp. Univ. Japan **38** (Pl. 3): 344, 1937.

Amphigenous; mycelium evanescent; perithecia scattered to gregarious, subglobose, 90 – 133μ in diameter; cells irregular in shape, obscure; appendages 12 – 40, 1 – 2 times the diameter of the perithecia, hyaline, straight, refractive, aseptate, thick-walled below, smooth, apex uncinate; asci subglobose to broadly ovate, sessile to shortly stalked, 39 – 54 x 32 – 36μ; Asco -spores 3 – 6, more or less crowded in the ascus, oblong to elliptical, 22 – 25 x 10 – 13μ.

On living leaves of *Celtis* sp. (*Urticaceae*), H.C.I.O. No. 27038, Kulu, Punjab, 29–11–57 (H. S. Gill).

*Podosphaera oxyacanthae* (DC.) de Bary in Beitr. Morph. u Phys. Pilz. **1**: 48, 1870; Homma, Jour. Hokk. Imp. Univ. Japan **88** (Pl. 3) : 318, 1937.

Hypophyllous; mycelium evanescent; perithecia scattered to gregarious in clinging masses, subglobose, 65 – 83μ in diameter; cells 10 – 18μ wide, appendages 4 – 12, spreading, 1 – 2 times the diameter of the perithecia; septate, dark brown for more than half their length, apex 2 – 4 times dichotomously branched, branches short and equal, ultimate branchlets rounded; ascus broadly obovate or sub-globose, 58 – 65 x 54 – 61μ; Asco-spores 6 –8, usually 8, measuring 18 – 22 x 11 – 13μ.

On living leaves of *Spiraea* sp. (*Rosaceae*), H C I.O. No. 27039, Kulu, Punjab, 29–10–58 (H. S. Gill).

*Uncinula mori* Miyake; Bot. Mag. Tokyo **21** : 1, 1907; Homma, Jour. Hokk. Imp. Univ. Japan **38** (Pl. 3): 366, 1937.

Epiphyllous; mycelium sub-persistent to evanescent; perithecia scattered to gregarious, 90 – 108μ in diameter, depressed-globose to globose; cells 11 – 18μ wide; appendages 12 – 20, simple, aseptate, hyaline, about equal or slightly longer than the perithecia, flexuous, bent at the upper portion, apex uncinate; asci globose, broadly ovate, with short stalk, 54 – 61 x 40 – 47 μ; Asco-spores 4 – 6, elongate-ellipsoidal, 22 – 29 x 14 – 15.5μ

On living leaves of *Morus* sp. (Moraceae), H.C.I.O. No. 27040. Katrain, Kulu Valley, Punjab, 15–11–59 (H. S. Gill and Gian Singh).

We are grateful to Dr. R. S. Vasudeva Head of the Division of Mycology and Plant Pathology for his keen interest helpful criticism and encouragement. Our thanks are also due to Mr. J. N. Kapoor, Herbarium Keeper for help in the determination of some of the species and their hosts and to Rev. Fr. Dr. H. Santapau for kindly rendering the latin diagnosis of the new species.

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# SOME CERCOSPORA SPECIES FROM INDIA-VI

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(Accepted for publication July 30, 1961)

In this paper, which is the fifth of the series, are included records of 19 species from India, of which 7 are new species and 2 new varieties. A number of species have economic hosts such as *Phaseolus aconitifolius*, *Eleusine coracana*, *Guizotia abyssinica*, *Prunus communis*, *P. persica* and *P. domestica*, *Cydonia oblonga*, and *Mangifera indica* which may be of particular interest to the Plant Pathologists. The specimens have been deposited in the Herb. Crypt. Ind. Orient., I.A.R.I., New Delhi and their respective numbers are indicated in the text.

*Cercospora alysicarpi* Munjal, G. Lall & Chona, Indian Phytopath. **12**: 131, 1959.

On living leaves of *Alysicarpus monilifer* DC. (Leguminosae), I.A.R.I., New Delhi, 6–11–1948, G. Lall, H.C.I.O., No. 26867.

The fungus agrees with the above species, already described on *Alysicarpus* sp., the present report is just a new host record.

*Cercospora anaphalidis* sp. nov.

Foliorum maculae subcirculares vel irregulares, 1–7 mm. diam., dispersae vel nonnumquam confluences, fusce brunneae; fructificationes amphigenae sed vulgo in superiore pagina foliorum; stromata tenuia, constantia e paucis cellulis fusce brunneis; fasciculi non densi, divergentes; conidiophori olivaceo-brunnei, septati, non ramosi, geniculati, nonnumquam pallidi ad apices, cicatrice sporarum eminente, 5 – 6 x 40 – 133$\mu$; conidia hyalina, vel subhyalina, acicularia vel nonnulla anguste obclavata, distincte et sat arcte septata, recta vel curvata, fastigata supra, truncata vel subtruncata ad basim, acuta vel subacuta ad apicem, 3 – 6 x 16 – 186$\mu$.

In foliis viventibus *Anaphalidis* sp. e familia Compositarum, ad Ranikhet, in Kumaon, U.P., die 16 octobris anni 1959, Leg. J. N. Kapur, H.C.I.O. sub numero 26853, Typus.

Leaf spots subcircular to irregular, 1 –7 mm. in diameter, scattered or sometimes confluent, dark brown in colour; fruiting amphigenous but mostly on the upper side of the leaf; stromata slight, consisting of a few dark cells; fascicles not dense, spreading; conidiophores olivaceous brown, septate, not branched, geniculate, tip sometimes dilutely coloured, spore-scar prominent, 5 –6 x 40 – 133$\mu$; conidia hyaline to subhyaline, acicular some narrowly obclavate, straight to curved, tapering above, base truncate to subtruncate, tip acute to subacute, septa distinct and some-what close measuring 3 – 6 x 16 – 186$\mu$ (Fig. 1).

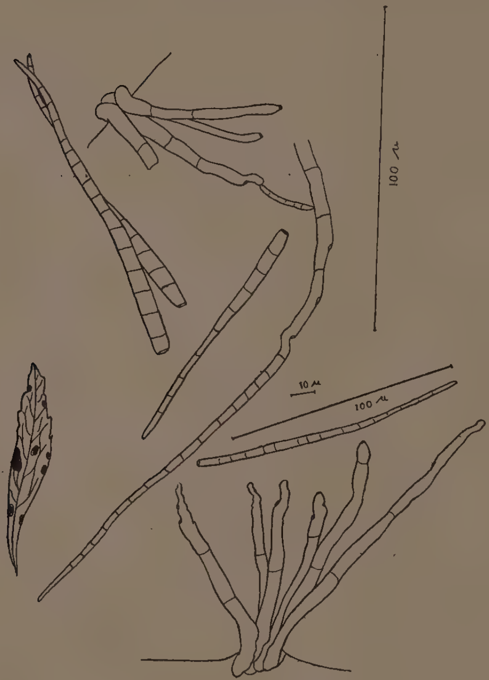


Fig. 1. *Cercospora anaphalidis*

On living leaves of *Anaphalis* sp. (Compositae), Ranikhet, Kumaon, (U.P.), 15–10–1959, J. N. Kapoor, H.C.I.O., No. 26853.

*Cercospora circumscissa* Sacc., Nuov. Giron. Bot. Italy **8** : 189, 1876; Sacc. **4** : 460, 1886.

On living leaves of *Prunus communis* Hud. (Rosaceae), Gurdaspur, (Punjab), 25–11–1959, R. L. Munjal, H.C.I.O., No. 26866.

The fungus forms spots which are reddish-brown to grey in the centre with black margin, bearing fructifications, generally on both surfaces of the leaf. The conidiophores arise from the stromata in fascicles, are olivaceous brown, septate, nodulose and measure 3 – 4 x 25 – 68μ. The conidia are pale-olivaceous, obclavate, septate, tapering above and measure 3 – 5 x 43 – 81μ.

*Cercospora columaris* Ell. & Ev., Proc. Acad. Nat. Sci. Phila. **46**: 380, 1894; Sacc. **11** : 625, 1895.

On living leaves of *Phaseolus aconitifolius* Jacq.. (Leguminosae), Pusa, (Bihar), 24–9–1930, U.B. Singh, H.C.I.O., No. 26860.

The fungus forms numerous angular spots, olivaceous in colour, which coalesce and cover large areas. Fructifications on both surfaces of the leaf but comparatively more on the lower surface which are olivaceous to grey and often effuse. The conidiophores arise from small stromata in fascicles, are brown, septate, not branched and measure 4 – 6 x 31 – 231µ. The conidia are hyaline to subhyaline, cylindric, septate and measure 4 – 5 x 30 – 77µ.

*Cercospora cydoniae* Ell. & Ev., Jour. Mycol. **8** : 72, 1902; Sacc. **18** : 601, 1906.

On living leaves of *Cydonia oblonga* Mill. (Rosaceae), Horticulture area, I.A.R.I., New Delhi, 1–11–1958, Ved Prakash and Gian Singh, H.C.I.O., No. 26854.

The fungus forms spots which are dark purple to dark brown, bearing fructifications mostly on the upper surface of the leaf. Conidiophores arising from well-developed stromata in dense fascicles, are olivaceous brown, simple, short and measure 2 – 3 x 6 – 22µ. Conidia narrowly obclavate, pale olivaceous, septate and measure 2 – 3 x 31 – 68µ.

*Cercospora diospyricola* sp. nov.

Foliorum maculae subcirculares vel irregulares, 2 – 13 mm. diam., nonnumquam confluentes, marginibus indefinitis, fulvae vel badiae; fructificationes amphigenae, sed vulgo in superiore pagina foliorum; stromata diametientia nonnullas cellulas ad 38.5µ, subglobosa, fusce brunnea; fasciculi densi; conidiophori olivaceo-brunnei, fusci in massa, septati, non ramosi, vix geniculati, sinuosi, irregulares latitudine, 4 – 6 x 39 – 92µ; conidia obclavata, raro cylindrica, subhyalina vel pallida, recta vel curvata, obconice longo-subtruncata ad basim, obtusa ad apicem, 4 – 5 x 15 – 77µ.

Typus lectus in foliis viventibus *Diospyri* e familia Ebenacearum ad Kathgodam, in Nainital in provincia U.P. die 23 octobris anni 1959 a J. N. Kapoor et positus in Herb. Crypt. Indiae Orient. sub numero 26864.

Leaf spots subcircular to irregular, 2 – 13 mm. in diameter, sometimes confluent, margin indefinite, fulvus to badius in colour; fruiting amphigenous but mostly on the upper side of the leaf; stromata few cells to 38.5µ in diameter, subglobose, dark brown; fascicles dense; conidiophores olivaceous brown, dark in mass, septate, not branched, sparingly geniculate, sinuous, irregular in width, measuring 4 – 6 x 39 – 92µ; conidia obclavate, rarely cylindric, subhyaline to pale, straight to curved, septate, base obconically long and subtruncate, tip obtuse, 4 – 6 x 15 – 77 µ (Fig. 2).

On living leaves of *Diospyros* sp. (Ebenaceae), Kathgodam, Nainital (U.P.), 23–10–1959, J. N. Kapoor, H.C.I.O., No. 26864.

*Cercospora eleusinis* sp. nov.

Maculae elongatae, in foliis et foliorum vaginis, 0.5 – 2.0 x 2.0 – 8.0 mm. diam., olivaceo-brunneae vel fusce brunneae ad margines, centro

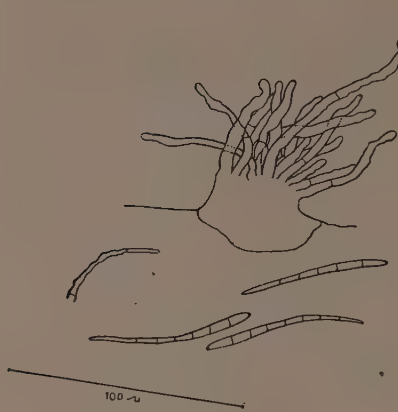


Fig. 2. *Cercospora diospyricola*

griseo-albo. Fructificationes hypophyllae. Stromata subglobosa fusce brunnea, minuta, ad 58.0 diam. Fasciculi rari vel densi, divaricati. Conidiophori olivaceo-brunnei, pallidi ad apices, recti vel curvati, geniculati, septis longiore intervallo separatis, non ramosi, cicatricibus sporarum eminentibus, 4 – 5 x 27 – 300μ; conidia acicularia, hyalina vel subhyalina, indistincte multiseptata, recta vel curva, truncata ad basim, acuta vel subacuta ad apicem, 3 – 4 x 50 – 260μ.

Typus lectus in foliis et foliorum vaginis *Eleusinis coracanae* Gaertn. e familia Graminearum, ad Kathgodam, Nainital in U.P. die 23 octobris anni 1959 a J. N. Kapoor et positus in Herb. Crypt. Ind. Orient. sub numero 26848.

Spots elongate on leaves and leaf-sheaths, 0.5 – 2 x 2 – 8 mm., olivaceous brown to dark brown margin with greyish white centre; fruiting hypophyllous; stromata subglobose, dark brown, small to 58μ in diameter; fascicles few to dense, spreading. Conidiophores olivaceous brown, tip dilutely coloured, straight to cuved, geniculate, septations at long intervals, not branched, spore-scar prominent; measuring 4 – 5 x 27 – 300μ. Conidia acicular, hyaline to subhyaline, indistinctly multiseptate, straight to curved, base truncate, tip to subacute, and measure 3 – 4 x 50 – 260μ (Fig. 3).

On living leaves and leaf-sheaths of *Eleusine coracana* Gaertn. (Gramineae), Kathgodam, Nainital (U.P.), 23–10–1959, J. N. Kapoor, H.C.I.O., No. 26848.

Venkatarayan (Mysore Agric. J. **24**(2) : 55, 1946) reported a spot disease, caused by a *Cercospora* species, on this host from Mysore, but he did not make the specific determination.

*Cercospora gerberae* Chupp & Viegas, Bol. da Soc. Brasil. de Agron. **8** : 27, 1945.


Fig. 3. *Cercospora eleusinis*

On living leaves of *Gerbera* sp. (Compositae), I.A.R.I., New Delhi (Delhi), 31–8–1943; A. Khan, H.C.I.O., No. 26852.

The fungus forms dark brown spots with darker margins, bearing fructifications on both the surfaces but chiefly on the upper side of the leaf. The conidiophores are pale to brown, geniculate, sparingly septate and measure 4 – 5 x 8 – 79μ. The conidia are hyaline, acicular, septate and measure 3 – 4 x 46 – 108μ.

*Cercospora guizotiae* Siemaszko, Mat. Mikol. i Fitopatol. Ross. **1**(3): 40, 1915; Sacc. **25** : 872, 1931.

On living leaves of *Guizotia abyssinica* Cass. (Compositae), I.A.R.I., New Delhi (Delhi), 3–11–1959, G. Lall, H.C.I.O., No. 26851.

On the leaves, the fungus produces spots which are fulvus with dark purple margin, bearing fructifications on both the surfaces. Conidiophores arising out of stromata in fascicles, brown, septate, geniculate, sometimes tuberculate at the base and measure 5 – 6 x 31 –127μ. Conidia acicular, hyaline, septate measuring 4 – 5 x 31 – 154μ.

*Cercospora hamiltoniae* sp. nov.

Foliorum maculae circulares vel subcirculares, 1 – 7 mm. diam., dispersae vel nonumquam coalescentes, nervis limitatae, griseolae in medio, marginibus fuscis; fructificationes amphigenae; stromata brunnea, cellulis paucis ad 37μ diam., fasciculi non densi, divergentes; conidiophori olivaceo-brunnei, septati, sprase geniculati, nonnumquam bulbosi ad basim, non ramosi, nonnulli ad septa constricti, irregulares latitudine, 5 – 7 x 40 – 105μ, bulbose quidem usque ad 9μ ad basim; conidia pallida, anguste obclavata,

cylindrica cum parva, septata, recta vel curva, fastigata supra, obconice truncata ad basim, subacuta ad apicem, 2 – 5 x 22 – 59μ.

In foliis viventibus *Hamiltoniae* spec. e familia Rubiacearum ad Govt. Sunder Nursery, New Delhi, die 23 decembris, legit G. Lall, H.C.I.O. sub numero 26850.

Leaf spots circular to subcircular, 1 – 7 mm. in diameter scattered or sometimes coalescing, vein-limited, greyish in cetnre with dark margin; fruiting amphigenous; stromata brown, few cells to 37μ in diameter; fascicles not dense, divergent; conidiophores olivaceous brown, septate, sparingly geniculate, not branched, a few constricted at the septa, irregular in width, sometimes bulbous at the base and measure 5 – 7 x 40 – 105μ, when bulbous up to 9.0μ in diameter; conidia pale, narrowly obclavate, cylindric when small, septate straight to curved, tapering above, base obconically truncate, tip subacute, measuring 2 – 5 x 22 – 59μ (Fig. 4).

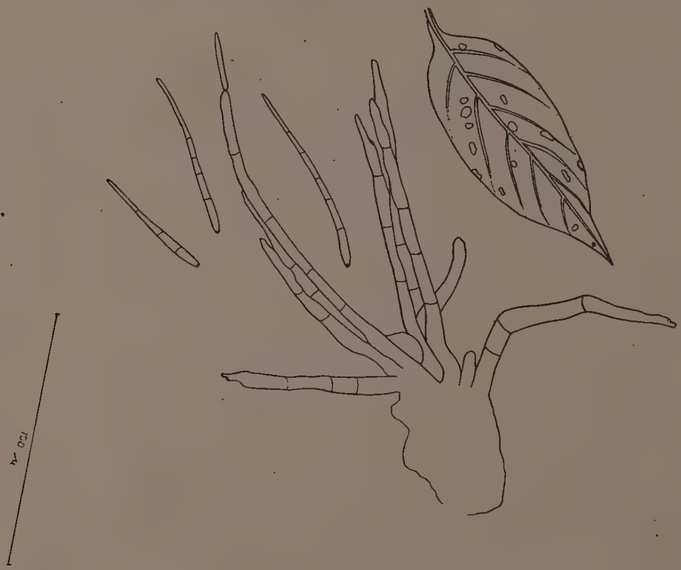

Fig. 4. *Cercospora hamiltoniae*

On living leaves of *Hamiltonia* sp. (Rubiaceae), Govt. Sunder Nursery, New Delhi (Delhi), 23–12–1954, G. Lall, H.C.I.O., No. 26850.

*Cercospora kaki* Ell. & Ev., Jour., Mycol. **3** : 17, 1887; Sacc. **10** : 643, 1892.

On living leaves of *Diospyros montana* Roxb., Mathura Road, New Delhi (Delhi), 23–4–1954, G. Lall, H.C.I.O., No. 26862; of *D. tomentosa* Roxb., Saharanpur (U.P.), 3–10–1955, J. N. Kapoor, H.C.I.O. No. 26849; of *D. virginiana* L. (Ebenaceae), Saharanpur (U.P.), 13–10–1954, M. L. Seth, H.C.I.O., No. 26840.

The fungus forms spots which are rusty brown surrounded by dark narrow margin but on *D. tomentosa*, the spots have greyish white centre and bear fructifications mostly on the upper surface of the leaf. Conidiophores arising from well developed stromata in fascicles, very short, pale, simple and measure 3 – 4 x 8 – 12μ. Conidia filiform to narrowly obclavate, sub-hyaline to pale, straight to curved measuring 3 – 4 x 30 – 73μ.

*Cercospora longissima* Sacc. var. *indica* var. nov.

Macuale circulares vel irregulares, coalescentes in maculas largiores; fructificationès amphigenae, effusae, fusce olivaceae; stromata nulla vel minuta; fasciculi non densi, patentes; conidiophori olivaceo-brunnei, septati, non ramosi, non geniculati, pallidi ad apicem, 4 – 6 x 39 – 116μ; conidia obclavato – cylindrica, subhyalina vel pallida, 0 – 6 – septata, obconice truncata ad basim, obtusa vel subobtusa ad basim, 5 – 8 x 19 – 77μ.

In foliis viventibus *Lactucae* spec. e familia Compositarum ad Pusa in Prov. Bihar, die 24 septembris anni 1930 a U. B. Singh et R. Nath, H.C.I.O. sub numero 26859.

Spots circular to irregular, coalescing to form large patches; fruiting amphigenous, effuse, dark olivaceous; stromata none or small; fascicles not dense, spreading; conidiophores olivaceous brown, septate, not branched, not geniculate, tip dilutely coloured, 4 – 6 x 39 – 116μ; conidia obclavato-cylindric, subhyaline to pale, 0–6 septate, base obconically truncate, tip obtuse to subobtuse, 5 – 8 x 19 – 77μ. (Fig. 5).

On living leaves of *Lactuca* sp. (Compositae), Pusa (Bihar), 24–9–1930, U. B. Singh and R. Nath, H.C.I.O, No. 26859.

*Cercospora mangiferae-indicae* sp. nov.

Foliorum maculae subcirculares vel irregulares, 1 – 3.5 mm. diam., dispersae vel confluentes, cinerae in medio, marginibus fusce brunneis in pagina superiore, in inferiore vero umbrinae in medio, marginibus fuscis; fructificationes amphigenae sed frequentiores in superiore pagina foliorum; fasciculi pauci; stromata minuta, constantia e cellulis nonnullis fusce brunneis; conidiophori olivaceo-brunnei, septati, geniculati, non ramosi, irregulares latitudine, dilute colorati ad apices, sporarum cicatricibus vix eminentibus, 3 – 6 x 16 – 115μ; conidia hyalina vel subhyalina, acicularia, septata, recta vel curva, fastigata supra, truncata ad basim, acuta vel subacuta ad apices, 3 – 6 x 22 – 180μ.

In foliis viventibus *Mangiferae indicae* L. e familia Anacardiacearum, Indian Agricultural Research Institute, New Delhi, leg. V. Prakash, H.C.I.O. sub numero 26847 Typus.

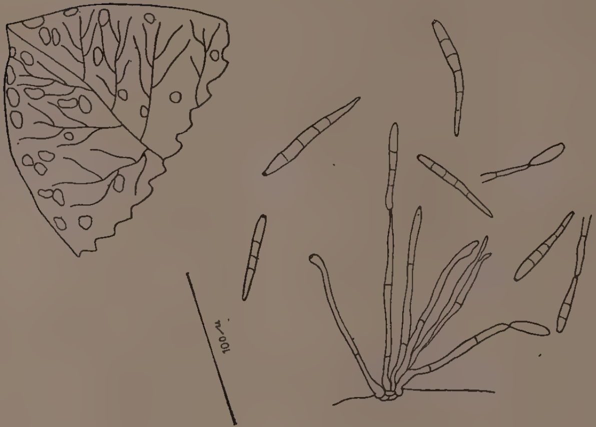


Fig. 5. *Cercospora longissima* var. *indica*

Leaf-spots subcircular to irregular, 1 mm. to 3.5 cm. in diameter, scattered or confluent, ash-coloured in the centre with brown margin on the upper surface, and umber in the centre with dark margin on the lower surface; fruiting amphigenous but chiefly on the upper surface; fascicles few; stromata small, consisting of a few dark brown cells; conidiophores olivaceous brown, septate, geniculate, not branched, irregular in width, tip dilutely coloured, spore-scar prominent, 3 – 6 x 16 – 115μ; conidia hyaline to subhyaline, acicular, septate, straight to curved, tapering above, base truncate, tip acute to subactue, 3 – 6 x 22 – 180 μ (Fig. 6).

On living leaves of *Mangifera indica* L. (Anacardiaceae) 11–12–1959, Indian Agricultural Research Institute, New Delhi (Delhi), Ved Prakash, H.C.I.O., No. 26847.

*Cercospora medicaginis-lupulinae* sp. nov.

Foliorum maculae indistinctae; fructificationes in maculis effusis fuscis in pagina inferiore foliorum, et raro etiam in pagina superiore; stromata minuta vel nulla; fasciculi pauci; condiophori olivaceo-brunnei vel ferruginei, septati, sparse geniculati, non ramosi, nonnumquam pallidi ad apices, 4 – 5 x 23 – 92μ; conidia pallida, cylindrico-obclavata, 2 – 5-septata, vulgo curva, obconice truncata ad basim, obtusa vel subobtusa ad apicem, 5 – 7 x 23 – 42μ.

In foliis viventibus *Medicaginis lupulinae* Linn. e familia Leguminosarum, ad Dehra Dun, U.P., die 22 septembris anni 1905, leg. Inayat Khan, H.C.I.O. sub numero 26861 Typus.

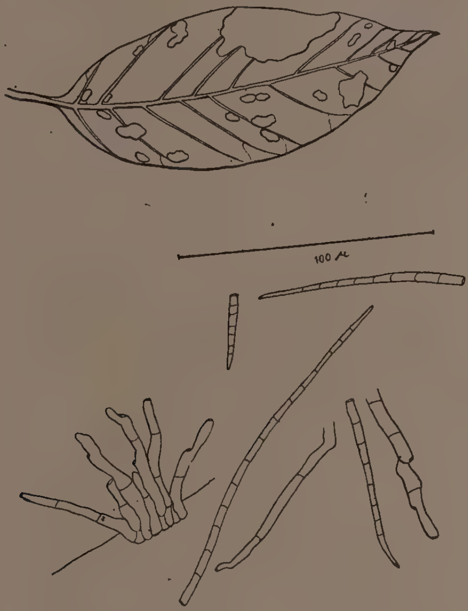


Fig. 6. *Cercospora mangiferae-indicae*

Leaf-spots indistinct; fruiting in dark effuse patches on the lower leaf surface and rarely on the upper surface also; stromata small or none; fascicle few; conidiophores olivaceous brown to ferrugineus, septate, sparingly geniculate, not branched, sometimes tip dilutely coloured, 4 – 5 x 23 – 92µ; conidia pale, cylindro-obclavate, 2 – 5 septate, mostly curved, base obconically truncate, tip obtuse to subobtuse, 5 – 7 x 23 – 42 µ (Fig. 7).

On living leaves of *Medicago lupulina* L. (Leguminosae), Dehra Dun (U.P.), 22–9–1905, Inayat Khan, H.C.I.O., No. 26861.

*Cercospora pachyderma* H. & P. Syd. var. *indica* var. nov.

Maculae indefinitae, effusae in pagina inferiore, olivaceo-brunneae vel nigrae; fructificationes hypophyllae; stromata constantia cellulis nonnulliis ad 58 µ diam., globosa vel subglobosa, fusce brunnea; fasciculi rari vel densi, divergentes; conidiophori olivaceo-brunnei, septati, geniculati, aliquantum ad septa constricti, nonnumquam bifurcati ad apices, pallide colorati, 4 – 6 x 42 – 108µ; conidia acicularia vel anguste obclavata, nonnulla raro cylindrico-obclavata, hyalina vel subhyalina, septata, recta vel curva, ad basim obconice truncata vel simpliciter truncata, acuta vel subobtusa ad apicem, 2 – 4 x 15–100µ.


Fig. 7. *Cercospora medicaginis lupulinae*

In foliis viventibus *Dioscoreae* sp. e familia Dioscorearum ad Jeolikote, Kumaon, leg. J. N. Kapoor, 22 decembris 1959, H.C.I.O. sub numero 26873 Typus.

Spots indefinite with effuse growth on the under surface of the leaf, olivaceous to black in colour; fruiting hypophyllous; stromata from a few cells to 58µ in diameter, globose to subglobose, dark brown; fascicles few to dense, spreading; conidiophores olivaceous brown, septate, geniculate, somewhat constricted at the septa, sometimes tip bifurcated and dilutely coloured, 4 – 5 x 42 x 108µ; conidia acicular to narrowly obclavate, some rarely cylindro-obclavate, hyaline to subhyaline, septate, straight to curved, base truncate to obconically truncate, tip acute to sub obtuse, 2 – 4 x 15 – 100µ (Fig. 8).


Fig. 8. *Cercospora pachyderma* var. *indica*

On living leaves of *Dioscorea* sp. (Dioscoreae), Jeolikote, Kumaon (U.P.), 22–10–1959, J. N. Kapoor, H.C.I.O., No. 26873.

*Cercospora pogostemonis* sp. nov.

Maculae foliorum circulares vel aliquantum irregulares, 10 – 15 mm. diam., brunneolae, evadentes olivaceae; fructificationes amphigenae; stromata minuta; fasciculi pauci; conidiophori fumosi, simplices, non ramosi, septis et geniculationibus nullis, 3 – 6 x 8 – 31$\mu$; conidia cylindrica vel anguste obclavata, indistincte multiseptata, vulgo curva, truncata vel rotundata ad basim, obtusa vel subobtusa ad apicem, 3 – 5 x 15 – 77$\mu$.

In foliis viventibus *Pogostemonis plectranthoidis* Desf. e familia Labiatarum ad Khandala in prov. Bombay, mense decembri anni 1954 leg. P. M. Verma, H.C.I.O. sub numero 26863 Typus.

Leaf-spots circular to somewhat irregular, 10 – 15 mm. in diameter, brownish turning olivaceous in due course; fruiting amphigenous; stromata small, brown; fascicles few; conidiophores smoky grey, simple, not branched, septation and geniculation lacking, 3 – 6 x 8 – 31$\mu$; conidia cylindric to narrowly obclavate, indistinctly many septate, mostly curved base subtruncate to rounded, tip obtuse to subobtuse, 3 – 5 x 15 + 77$\mu$ (Fig. 9).

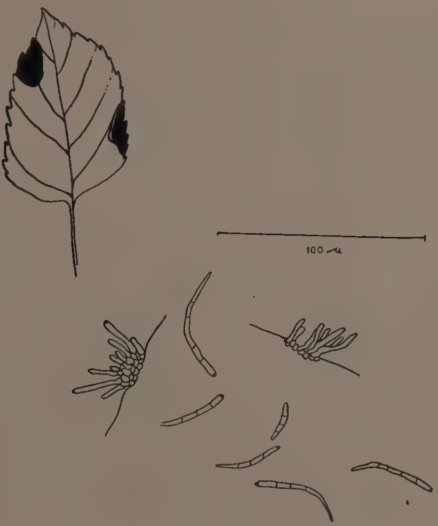


Fig. 9. *Cercospora pogostemonis*

On living leaves of *Pogostemon plectranthoides* Desf. (Labiatae) Khandala (Bombay), Dec. 1954, P. M. Verma, H.C.I.O., No. 26863.

*Cercospora prunicola* Ell. & Ev., Jour. Mycol. **3** : 17, 1886; Sacc. **10** : 643, 1892.

On living leaves of *Prunus persica* (L.) Batsch., Gurdaspur (Punjab), 25–11–1959, R. L. Munjal, H.C.I.O., No. 26865; of *P. persica* (L.) Batsch. var. *gujrati*, Horticulture area, Indian Agricultural Research Institute, New Delhi (Delhi), 1–11–1959, Gian Singh and Ved Prakash, H.C.I.O., No. 26858; of *P. domestica* L. (Rosaceae), Horticulture area, Indian Agricultural Research Institute, New Delhi (Delhi), 1–11–1959, Gian Singh and Ved Prakash, H.C.I.O., No. 26857.

Leaf spots dark brown, bearing fructifications mostly on the lower surface. Conidiophores arising from well developed stromata, pale olivaceous brown, simple, short, not branched and measure 3 – 4 x 15 – 43μ. Conidia are narrowly obclavate, sometimes smaller cylindric, pale, septate and measure 2 – 3 x 22 – 102μ.

*Cercospora sphaeroidea* Speg., Anal. Soc. Scient. Argentine **16** : 169, 1883; Sacc. **4** : 463, 1886.

On living leaves of *Cassia auriculata* L. (Leguminosae) Devkamota (Madras), 2 –1–1959, K. R. Sreekantiah, H.C.I.O., No. 26856.

On the leaves, the fungus produces spots which are grey on the upper surface and tan coloured on the lower, bearing fructifications on both the surfaces. The conidiophores come out of well developed stromata in fascicles are olivaceous brown, sparingly geniculate, septate, not branched and measure 4 – 6 x 12 – 43μ. The conidia are olivaceous brown, cylindro-obclavate, 2 – 6 septate and measure 5 – 6 x 22 – 49μ.

*Cercospora varia* Peck, N. Y. State Mus. Ann. Rept. **35** : 141, 1884; Sacc. **4** : 468, 1886.

On living leaves of *Viburnum coriaceum* Bl. (Caprifoliaceae), Ranikhet, Kumaon, (U.P.), 16–10–1959, J. N. Kapoor, H.C.I.O. No. 28855.

On the leaves, the fungus forms spots which are brick coloured surrounded by tan coloured margin, bearing fructifications on both the surfaces. The conidophores arise from well developed stromata in fasicles which are olivaceous brown to dark brown, septate, geniculate, rarely branched, variously curved and measure 4 – 5 x 22 – 144μ. The conidia are colourless, cylindo-obclavate, septate and measure 4 – 5 x 14 – 43μ.

Grateful thanks are due to Dr. R. S. Vasudeva, Head of the Division of Mycology and Plant Pathology and Joint Director, for his keen interest, encouragement and valuable criticism. We are also indbted to Rev. Fr. Dr. H. Santapau, Head of the Biology Department, St. Xavier's College, Bombay for rendering the latin diagnosis of new species and varieties and to Shri J. N. Kapoor, Herbarium-Keeper, for help in the identification of some species.

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# SPORE GERMINATION IN COLLETOTRICHUM FALCATUM WENT

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While working with different strains of *Colletotrichum falcatum* (*Glomerella tucumanensis* (Speg.) Arx and Müller), the causal organism of red rot of sugarcane, it was observed that the light type isolate (No. 244 of *C. falcatum* from the Indian Type Culture Collection) which was isolated from the red rot epidemic areas of Uttar Pradesh and has maintained its virulence for the last 14 years (Chona, 1954) showed repeatedly low germination in distilled water as compared to the dark strains which are genarally weakly parasitic. Low germination in this strain in distilled water was also reported by Chona and Srivastava (1954) and Bajaj, Ganju and Chatrath (1959). Abundant spore germination is an important character for the survival of a pathogen and its successful establishment on the host. In view of such an unusual association of apparently low germination and virulence it was considered necessary to study further the conditions which govern spore germination of the strain under consideration.

Material and Method: Single spore culture of virulent isolate No. 244 of *C. falcatum* maintained on oatmeal agar was used throughout for germination studies. For all the experiments in the initial stages, conidia from 10 to 20-day-old cultures grown at room temperature, were harvested, washed by centrifugation and concentration of spores in distilled water adjusted by means of a haemacytometer before putting up for germination. In order to avoid influence of any external source of nutrition, all the glassware including slides were washed with Sulphuric acid—Potassium dichromate mixture (1 litre acid: 60 ml. saturated solution of $K_2Cr_2O_7$) and distilled water before use. The volume of germination drop was kept constant in different experiments. Generally, four replicates were kept in each treatment and about 200 spores were counted in each drop. The slides were kept in moist chambers for 16 hours at 25°—26°C. The criterion of germination was the emergence of germ tube to a length exceeding the width.

Experimental Results: For the study of germination in distilled water different techniques, e.g., hanging drop method, cavity slide method, plain slide method, etc. were tried to study the germination of conidia. It was observed that germination varied considerably with the technique, maximum being 22.3 per cent in the case of hanging drop method. Plain and cavity slide methods showed germination from 1 to 15 per cent but there was considerable variation from drop to drop and, the results were, therefore, not strictly reproducible. The main draw-back was that the spores in this case being heavy tended to settle down. The germination was much greater at the periphery of the drop than in the centre. In the

case of plain slide method also, the germination of spores was greater at places where the germination drop had spread into a thin layer than in a formed drop. In the case of hanging drop method, all the spores got collected in the centre of the drop, due to their weight, and resulted also in low germination. The data of such tests are given below:

| *Method* | | | *Germination, Per cent* | | |
|---|---|---|---|---|---|
| | | | I | II | III |
| Plain Slide | ... | ... | 2.6 | 3.8 | 15.5 |
| Cavity Slide | ... | ... | 1.0 | 2.8 | 14.0 |
| Hanging drop | ... | ... | 15.3 | 22.3 | 18.7 |

In order to have a uniform size and volume of the germination drop, circles of different sizes, e.g., 1.2, 1.5 and 2.1 cm. diameter were marked on the plain slides with the aid of glass pencil, greese, collodion and euparol and equal volumes of spore suspension in distilled water deposited in each circle. Surprisingly, the germination of conidia in the circle of 2.1 cm. diameter marked with glass pencil increased to about 90 per cent and gradually decreased as the size of the circle was reduced. In the case of circles marked with greese, collodion and euparol, however, there was no germination which may be due to some toxic effects of the chemicals used. Later 'Cutex' was found to be more suitable, as glass pencil marks, apart from being a crude device, used to float in the germination drop after some time and thus affect the germination. The slides were marked with 'cutex' made by M/s Northan Warren Ltd., Lodnon atleast 24 hours* before use with the aid of a ringing table to get uniform size of the circle. Same ringed slides could be used for nearly 4–5 times for germination studies. With a view to determine the size of the circle in relation to the volume of the germination drop which might support maximum germination, circles of 1.3, 1.7 and 2.1 cm. diameter were marked and germination studied in distilled water using 0.03 ml. of spore suspension in each circle. Though the germination was maximum in 2.1 cm. diameter circle but the circle being too big for the volume of the suspension used, the germination, drop, after sometime, started receding and, therefore, the middle size circle (1.7 cm.) was considered suitable for further work.

In order to get reproducible results efforts were made to standardize most of the factors that influence spore germination, e.g. centrifugation of spores, temperature and period of incubation, concentration of spores in the germination drop, aeration, age of the culture, etc.

Physical Factors: It is observed from the results given below that washing of spores by centrifugation upto 10 minutes at 2,800 r.p.m. has practically no adverse effect on germination but centrifugation for longer periods, depresses the germination.

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*The Germination was markedly depressed if the freshly ringed slides were used.

| *Treatment* | *Germination, per cent.* |
|---|---|
| Unwashed spores ... ... | 86.3 |
| 10 minutes centrifugation ... | 81.7 |
| 20 ,, ,, ... ... | 35.8 |
| 30 ,, ,, ... ... | 5.1 |

Since washing of spores is essential for the germination studies to get consistent results, it was considered safe to centrifuge the spores once for 10 minutes at 2,800 r.p.m. Experiment conducted to show the period and temperature of incubation revealed that optimum temperature for germination in this organism is around 27 °C (Fig. 1) and that there is no germination at 6.6° and 40 °C. The spores could germinate at as low a temperature as 10 °C though the percentage germination was low. It was also observed that germination of conidia initiated after 6 hours of incubation at 27 °C (Fig. 2) and that it reached almost its maximum after 16 hours. Further incubation even for 48 hours did not appreciably increase germination percentage.

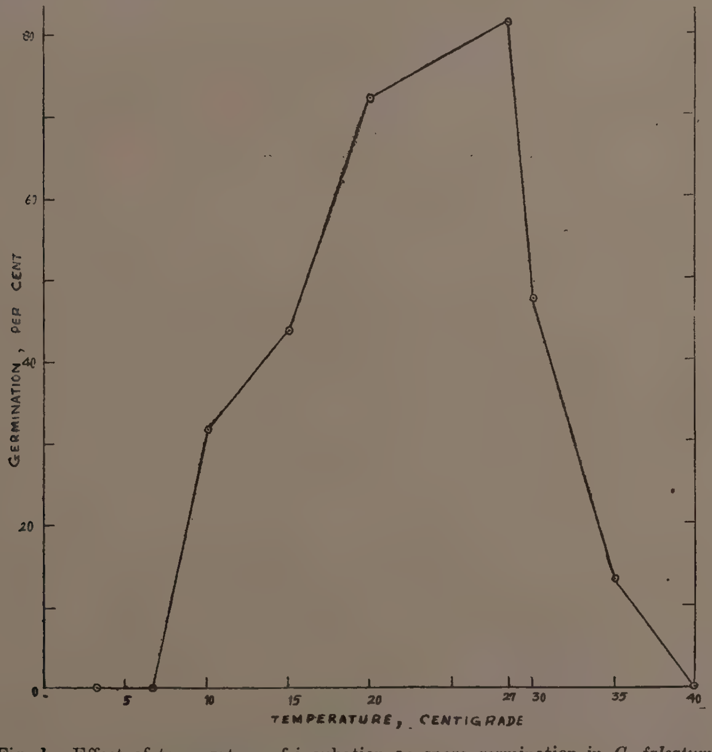


Fig. 1. Effect of temperature of incubation on spore germination in *C. falcatum*. Period of incubation 16 hours.

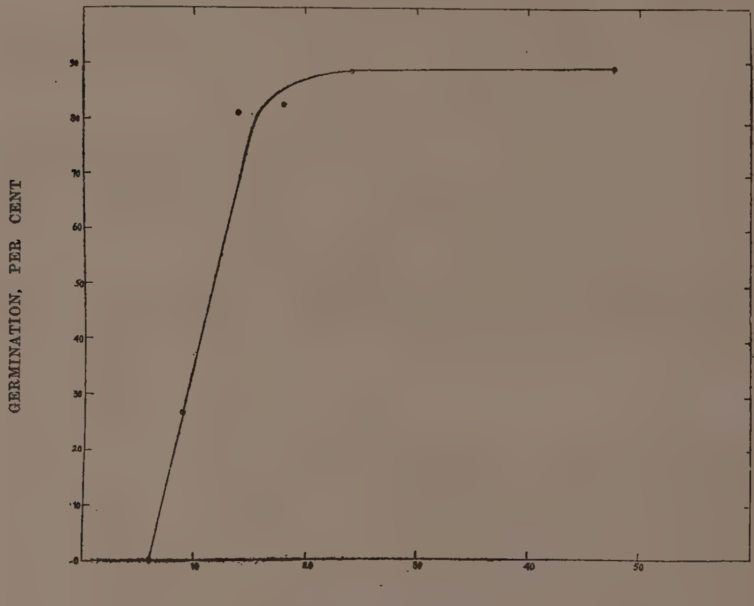


Fig. 2. Effect of incubation period on spore germination in *C. falcatum*. Temperature of incubation 27°C.

The experiment conducted to study the influence of hydrogen-ion concentration on germination using Sorsen's phosphate buffer (Clark, 1928) showed that optimum pH for germination of *C. falcatum* spores lies around 6.00. Like many other fungi, this organism also has an acidophilic character though the germination extends partly to the alkaline region. As is indicated from the results given below, the optimum range of pH for germination in this organism is rather narrow and therefore, pH is also one of the important factors for germination.

| *pH* | | | *Germination, per cent* |
|---|---|---|---|
| 5.5 | ... | ... | 68.6 |
| 6.0 | ... | ... | 80.8 |
| 6.5 | ... | ... | 23.7 |
| 7.0 | ... | ... | 11.0 |
| 7.5 | ... | ... | 6.5 |
| 8.0 | ... | ... | 0.0 |
| Distilled water (pH 6.1) | | ... | 82.3 |

*Age of the culture:-* The period between spore formation and their germination varies considerably in different organisms. This is also influenced by other factors, e.g., temperature, humidity, nutrition, etc. within the same organism. In the case of *C. falcatum*, cultures of different age were raised on oatmeal agar and incubated at 27°C. The germination

of conidia was studied in 5 to 30 - day-old cultures on the ringed slides by the method already described. The results of such an experiment are given in fig. 3.

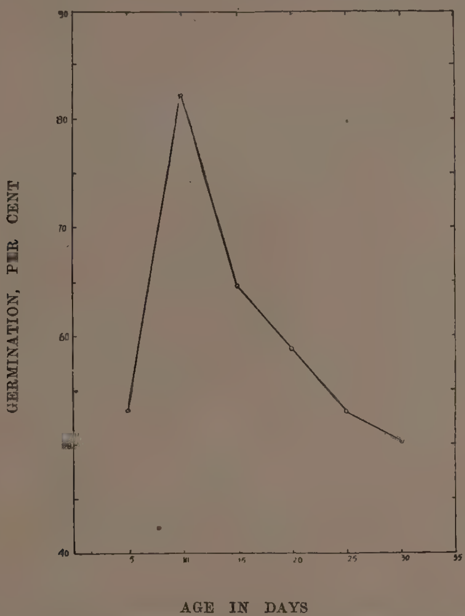


Fig. 3. Effect of age of culture on germination of spores in *C. falcatum*. Temperature of incubation 27 °C.

*Spore Concentration*:- Experiment to study the effect of spore concentration on germination in red rot organism was set up by adjusting the concentration of spores, after centrifugation, at optical density 0.27 (54.0 per cent transmission) by means of a Lumetron photoelectric colorimeter (Model S-48 A) using blue filter of 420 mμ wavelenght. This concentration was fixed as the standard stock concentration (designated as 'N') and different dilutions, e.g.. N/2, N/4, N/8, N/16, N/32 and N/64 were prepared in distilled water by serial dilution method. The results are presented in Fig. 4. The concentration at N/32 was considered optimum for germination as in the lower concentration, i. e. N/64 though there was slightly more germination, the number of conidia per drop was not adequate to give sizeable population for germination study.

With a view to study the self-inhibitory effect of spores in *C. falcatum*, if any, an experiment was set up by keeping the suspension of washed spores of 18-day-old culture in distilled water in 100 ml. Erlenmeyer flasks at room temperature for 6, 14, 24 and 32 hours at 0.27 optical denisity (N) concentration. Minimum period of six hours was chosen, as in earlier experiment visible germination was observed only after that period at N/32

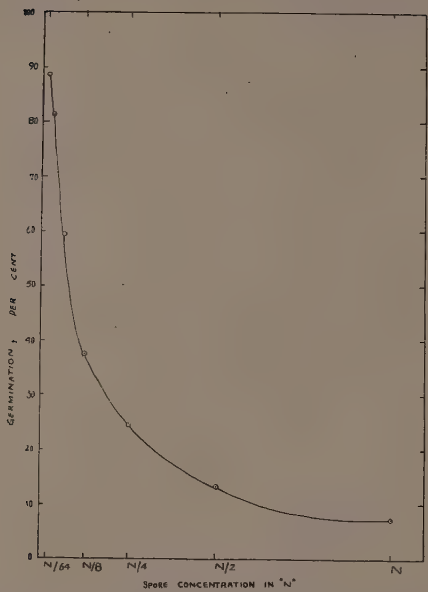


Fig. 4. Effect of spore concentration on their germination in *C. falcatum*. Concentration expressed in "N" where N = 0.27 O.D.

concentration. There was, however, no germination in any of the treatments in the concentrated suspension during the extraction period. The suspension was centrifuged and spore germination of 14-day-old culture was studied under optimum conditions in the supernatant, keeping control in distilled water. It was observed that germination was completely inhibited in all the treatments though control showed 80.6 per cent germination. In another experiment the supernatant of the concentrated suspension (N) of 18 and 21-day-old cultures kept only for 6 hours at room temperature was diluted by serial dilution method and germination of spores of 14-day-old culture studied under standard conditions. The results are given in table I.

In a variation from the above experiment, resuspended spores were centrifuged immediately, 15 minutes, 1, 4 and 6 hours after adjusting optical density at 0.27. The supernatant was used for studying germination of freshly washed spores. There was 13 to 15 per cent germination in the washings obtained from the first two treatments, i.e.., immediate and 15 minutes, whereas in the remaining treatments there was no germination except in the control which showed normal germination.

It appears that the reduction in spore germination in high concentration may be chiefly due to some self-inhibitory principle, the toxicity of

which could be reduced by dilution. Preliminary experiments with different isolates showed that the self-inhibition is probably not a general phenomenon with all the isolates as some isolates appeared to lose their capacity to secrete the toxic principle under certain cultural conditions. Further experimentation is, however, necessary to fully clarify this aspect.

Table I. Showing effect of self-inhibitory principle on spore germination.

| Concentration of the supernatant (per cent) | Germination, per cent | |
|---|---|---|
| | 18-day-old-culture | 21-day-old culture |
| 100.00 | 0.0 | 0.0 |
| 50.00 | 0.0 | 0.0 |
| 10.00 | 8.4 | 9.3 |
| 1.00 | 26.7 | 27.6 |
| 0.10 | 57.2 | 58.6 |
| 0.01 | 56.7 | 58.9 |
| Distilled water (control) | 80.6 | 82.0 |

In order to study the nature of the inhibitory principle, the supernatant diluted to 50 per cent was boiled and autoclaved at 15 lb. p.s.i. for 20 minutes and germination studied in comparison with unheated 50 per cent supernatant. There was no germination in any of the treatments showing, thereby, that the inhibitory principle is thermostable.

Aeration: Series of experiments were set up where depth, size, volume and the spore concentration of the suspension were varied in different combinations. The spore concentration of the germination drop was adjusted in such a manner that the concentration per unit area of the slide was always kept constant so as to avoid the interfering effect of self-inhibition. The results are given in table II.

In order to study the effect of available oxygen in the standard germination drop, 1.5 litres of distilled water was boiled in a flask for 45 minutes to remove the dissolved oxygen and cooled suddenly. Air was then blown in the boiled distilled water for 10, 15 and 20 minutes by means a suction apparatus connected with water tap, keeping controls in ordinary distilled and boiled distilled water. The germination of spores was studied by the usual method and the results are given below:

| Treatment | Germination, per cent |
|---|---|
| 1. Distilled water ... ... | 83.8 |
| 2. Boiled Distilled water ... | 57.5 |
| 3. Air blown for 10 minutes in boiled distilled water | 64.6 |
| 4. Air blown for 15 minutes in boiled distilled water ... ... | 72.3 |
| 5. Air blown for 20 minutes in boiled distilled water ... | 58.1 |

TABLE II. Effect of depth, size and volume of the drop on spore germination in *C. falcatum*.

| Constant Factor | Area of the Germination drop ($cm^2$) | Depth of the Germination drop (mm) | Volume of the germination drop (ml.) | Spore * concentration of suspension | Germination, per cent |
|---|---|---|---|---|---|
| Volume | 1.328 | 0.452 | 0.060 | N/109.48 | 42.1 |
| | 2.271 | 0.264 | 0.060 | N/64.00 | 59.7 |
| | 3.465 | 0.172 | 0.060 | N/41.90 | 69.8 |
| Area | 2.271 | 0.396 | 0.090 | N/96.00 | 44.1 |
| | 2.271 | 0.264 | 0.060 | N/64.00 | 59.7 |
| | 2.271 | 0.132 | 0.030 | N/32.00 | 80.5 |
| Depth | 1.328 | 0.264 | 0.035 | N/64.00 | 59.0 |
| | 2.271 | 0.264 | 0.060 | N/64.00 | 59.7 |
| | 3.465 | 0.264 | 0.091 | N/64.00 | 57.1 |

*Spore concentration (N) adjusted by colorimeter at optical density 0.27 and then dilutions prepared in such a way that number of spores per unit area was constant

It is observed that germination is reduced in the case of boiled distilled water as against ordinary distilled water. By blowing air into the boiled distilled water, the germination could be restored to about 72 per cent but further blowing of air appeared to depress germination which could be due to increased concentration of $Co_2$. In another experiment it was observed that double distilled water depressed the germination to about 10 per cent as against 81 and 91 per cent in ordinary distilled and tap water, respectively. In order to see whether the reduction in germination in double distilled water was due to depletion of oxygen or nutrients, air was blown in double distilled water for 15 minutes but it failed to effect improvement in germination. This obviously showed that some of the salts which could not be removed by first distillation, were depleted by the second distillation and thus the germination was adversely affected. This was further confirmed by setting up a germination experiment with de-ionized water obtained by using mixed-bed de-ionization technique (Samuelson, 1952) with ion exchange resins in comparison with distilled water. The de-ionized water (pH 7.00) showed no germination as against 80.6 per cent in the control.

DISCUSSION: Use of ringed slides for the study of spore germination, though a simple device, has its own usefulness particularly in fungi which have heavier spores and need good aeration for germination. Experiments conducted under controlled conditions have shown that with the volume as constant factor, the germination increases with decrease in depth

and increase in area of the germination drop but if the area is kept constant the germination increases with decrease both in depth and volume. However, if depth of the germination drop is kept uniform, the germination is unaffected even though the area and volume vary. These observations indicate that aeration is a factor of considerable importance in the germination. Unsatisfactory germination obtained on plain and cavity slides may be partly due to stringent aerobic conditions as the spores, being heavy, settle down whereas in the hanging drop self-inhibition of spores, due to their aggregation at the apex, would interfere in their germination. All the requisite requirements appear to have been met in the ringed slide method.

Maximum spore germination is obtained in 10-day-old cultures at 27 °C. Low germination in younger cultures may be due to immaturity of spores whereas the rapid fall in germination after it has reached its maximum may be due to senescence where the reserve nutrients within the spores tend to fall (Gottlieb, 1950). The period exhibiting maximum germination in *C. falcatum* is rather restricted (Fig. 4) so that the age of the spores is one of the critical factors in germination. The optima showing maximum germination, the stage of maturity and the senescence would, however, depend upon incubation temperature.

The nature of curve showing the influence of increased spore concentration on germination is exponential type and it may be possible to work out an equation with suitable constants to express the relation between spore concentration and germination.

Yarwood (1954, 1956) and Allen (1955) have experimentally demonstrated the production of toxic substances in spores of *Uromyces phaseoli* and *Puccinia graminis tritici*, which inhibit the germination when there is overcrowding and this has been explained on the basis of self-inhibition. Domsch (1954), working with *Erysiphe graminis* spores, showed that the greater the density of conidial suspension the lower the germination, which was due to the inhibitory effect of a substance excreted into the medium by the conidia themselves. Forsyth (1955) showed that the self-inhibition of germinating uredospores of *P. graminis tritici* was due to the production of trimethylethylene. On the other hand, Doran (1922) and Lilly and Barnett (1951) are of the opinion that this is due to the competition of the limited supply of oxygen rather than the toxic substances produced by germinating spores. In *C. falcatum* also the self-inhibitory substance is produced probably at the time of spore formation and inhibits the germination in high concentrations. The increase in germination at low concentrations seems to be due to the dilution of the inhibitor. The active principle inhibiting spore germination, apart from being heat-resistant, is obviously not volatile. Further experimentation is, however, necessary to study also the role of the limited supply of oxygen in suppressing germination in high concentrations as it is likely that both these factors, i.e. lack of oxygen and the self-inhibition, might be contributing towards this phenomenon.

The production of self-inhibitory principle in the spores of *C. falcatum* may have certain pathological value. The inocula in the form of spore

masses of red rot fungus, both on the cane and the mid-rib may be preserved due to the action of self-inhibitory principle and may be utilized in the dissemination of the disease under favourable conditions as the ungerminated spores which are chiefly responsible for the dispersal of the organism are more resistant to the adverse environmental conditions.

It is apparent that high conidial germination in distilled water obtained in the case of virulent strain of this organism is no exception to the rule even though the conditions for germination of spores are stringent. The conditions to meet the demand relating to spore germination are obviously met with in nature and adequate germination, which is a prerequisite to infection, does take place to perpetuate a virulent strain.

## Summary

A ringed-slide technique for the study of germination of spore of the light type isolate of *Colletotrichum falcatum* in distilled water has been described. It has been shown that spore concentration, age of the culture, pH, centrifugation and temperature markedly influence spore germination. Furthermore, the dimensions of the germination drop, particularly, the depth which directly influences the aeration of the drop, have been standardised. It has also been shown that at high concentration of the spores the germination is reduced due to self-inhibition.

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# NOTES ON SOME FUNGI FROM SOUTH INDIA

N. V. Sundaram*

(Accepted for publication July 30, 1961)

During the course of tours by the author to different parts of Madras State in 1955–56 a number of fungi were collected. Among them, those which were found to be new species or new records for India, are described in this note. The type specimens have been deposited in the Herbarium of the Government Mycologist, Coimbatore.

*Leveillula taurica* (Lev.) Arn. Saccardo, P.A., *Syll. Fung.*, **20**, 226, 1926.

On living leaves of *Passiflora edulis* Sims. (Passifloraceae), Coonoor, 22–11–55, N. V. Sundaram, G. M. Herb. No. 2868.

The infected leaves show irregular, pale yellow dis-colouration on the upper surface with whitish growth of the fungus on the corresponding lower surface.

*Crossopsora symphoremae* Sund. spec. nov. Pycnia and aecia were not observed. Uredia are amphigenous but predominently hypophyllous, minute, isolated or in groups formed below the epidermis. The urediospores are globose, oval or ellipsoid with echinulate wall, and yellowish brown contents, 25 x 19μ (19 – 40 x 15 – 22), wall coloured. The uredia are surrounded by marginal incurved paraphyses with 1 - 2 septa, light brown in colour, club shaped with irregularly thickened wall, 43–90 x 9 – 22μ. The telia are hypohyllous and formed in long tendril like columns, subepidermal in origin, paraphysate; the paraphyses being similar to those in the uredia. The telial column measures up to 5 mm. long and 95μ broad. The teliospores are sessile, single celled, oblong, vinaceous brown, 22 x 16μ (18 – 31 x 9 – 19), smooth and are strongly united together.

On living leaves of *Symphorema involucratum* Roxb. (Verbenaceae), Kallar (Coimbatore), 10–8–55, N. V. Sundaram, G. M. Herb. No. 2856 (Type).

*Crossopsora symphoremae* Sund. spec. nov. Pycnia atque aecia ignota; uredia ut plurimum hypophylla, rare epiphylla, minuta, singula vel aggregata, erumpentia, subepidermalia, pulverulenta; uredosporae globosae, ovatae vel ellipsoideae, 25 x 19μ (19 – 40 x 15 – 22), echinulatae, contentis luteo-brunneis, parietibus coloratis, paraphysatae; paraphyses marginales, incurvae, parietibus irregulariter crassis, maxime crassis ad apicem, 1 – 2 septatae, pallide brunneae, paraphysata; paraphyses ut in urediis; columna telialis 3–5 mm. longa, et usque ad 95μ lata; teliosporae sessiles, semel-cellulatae, unitae, oblongae, vinaceo-brunneae colore, 22 x 16μ (18 – 31 x 9 – 19), germinantes in situ ad maturitatem.

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Typus lectus in foliis viventibus *Symphorematis involucrati* Roxb. e. familia Verbenacearum, in loco Kallar, Coimbatore, die 10 mensis augusti anni 1952 a N. V. Sundaram, et positus in G. M. Herbario sub numero 2856.

Two rusts, viz., *Crossopsora premnae* (Petch) Syd. (1, 3) and *C. premnae-tomentosae* Ramakr. & Sowmini (2) have been recorded on the hosts *Premna corymbosa* and *P. tomentosa* (Verbenaceae) respectively. The present rust, although also occuring on a member of the family Verbenaceae but on a different host, viz., *Symphorema involucratum,* differs from the above two in the spore size, shape, colour of the spore wall and hence described as a new species.

Inoculations of the two hosts *Premna tomentosa* and *Symphorema involucratum* were carried out with urediospres of the respective rusts. It was found that each rust infected its own host only.

*Uredo garugae* Sund. spec. nov. Spots amphigenous, minute, yellowish brown with dark green margin; uredia hypophyllous, isolated or in groups, light orange in colour, paraphysate; paraphyses marginal, one celled, clavate, light brown; urediospores globose to subglobose, thin walled, echinulate, light brown, wall coloured, germpores 2, mostly found on the upper half of the spores, 22 x 16 $\mu$ (19 – 25 x 10 – 19).

On living leaves of *Garuga pinnata* Roxb. (Burseraceae), Wynaad, 15–9–55, N. V. Sundaram, G. M. Herb. No. 2874 (Type).

Maculae amphigenae, minutae, luteolo-brunneae, marginibus viridibus; uredia hypophylla, singula vel aggregata, pallide aurantiaca, paraphysata; paraphyses marginales, semel cellulatae, clavatae, pallide brunneae; urediosporae globosae vel subglobosae, tenuibus parietibus praeditae, echinulatae, pallide brunneae, parietibus coloratis, germinationis poro duplici ut plurimum in parte dimidia superiore sporarum posito, 22 x 16$\mu$ (19 – 25 x 10–19).

Typus lectus in foliolis viventibus *Garugae pinnatae* Roxb. e familia Burseracearum, in loco Wynaad, die 15 mensis septembris anni 1955, N. V. Sundaram, et positus in G. M. Herbario sub numero 2874.

Only the uredial stage of the rust was observed from the specimens examined. The infection is prominently seen by the presence of dark green areas surrounding the yellowish infected parts. The rust sori are formed on the middle of the spots. The colour of the uredia fades away on preservation.

*Uredo myriactidis* Sund. spec. nov. Spots epiphyllous, spherical, yellowish, varying in size, 2 to 5 mm. in diam; uredia hypophyllous, aggregated, subepidermal, epidermis long covered leaving a pore like opening and surrounded by a pseudoperidium; urediospores globose to subglobose, thin walled, echinulate, contents light orange, germpores not distinct, 19 x 16 $\mu$ (16 – 25 x 12 – 19), the stalks are short.


Fig. 1.. *Uredo garugae:* Section through uredium.

Fig. 2. *Uredo myriactidis:* Section of uredium showing the pseudoperidium and the urediospores.

Fig. 3 – 6. *Crossopsora symphoremae:* (3) Section of telium (4) Section of uredium (5) urediospores (6) germinating urediospore.

On living leaves of *Myriactis wightii* D.C. (Compositae). Doddabetta (Ootacamund), 19–11–55, N. V. Sundaram. G. M. Herb. No. 2876 (Type).

Maculae amphigeneae, sphaericae, luteolae, 2 – 5 mm. diam.; uredia hypophylla, aggregata, subepidermalia, epidermide intacta diu manente excepto foramine pori instar, quod pseudoperidio operitur. Urediosporae globosae vel subglobosae, tenuibus parietibus praeditae, echinulatae,

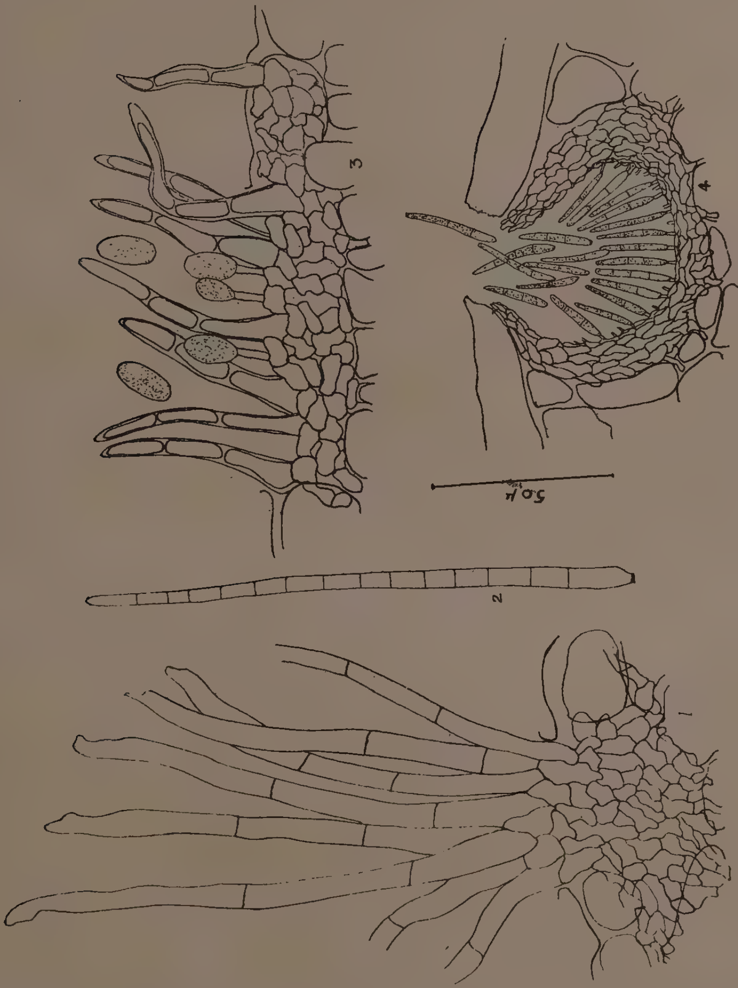


Fig. 1 – 2. *Cercospora duddiae:* (1) Section showing the stroma and conidiophores
(2) conidium.

Fig. 3. *Colletotrichum brachytrichum:* Section of an acervulus.

Fig. 4. *Septoria cycadis:* Section of a pycnidium.

contentis aurantiacis, germinationis poro indistincto, 19 x 16 μ (16–25x 12 – 19).

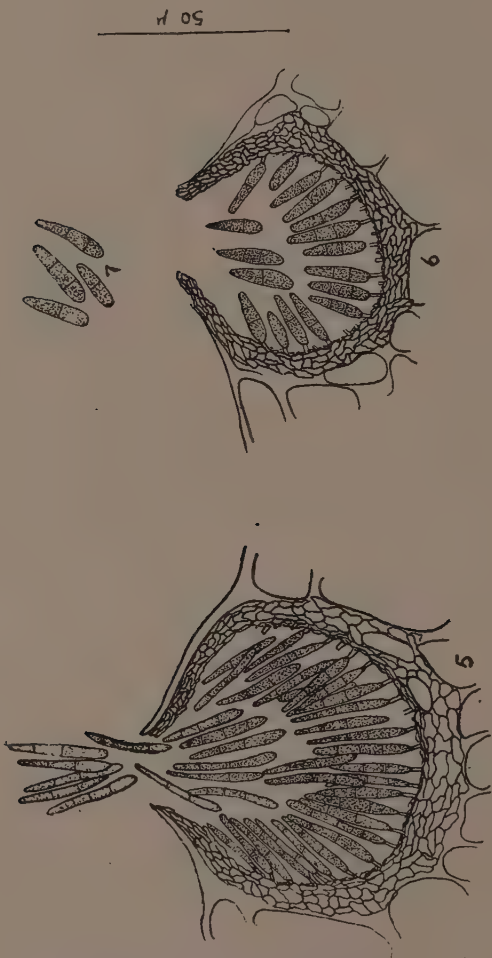


Fig. 5. *Septoria crotalariae:* Section of pycnidium.
Fig. 6 - 7. *Septoria zingiberis:* (6) Section of pycnidium (7) Pycnidiospores.

Typus lectus in foliis viventibus *Myriactidis wightii* DC. e familia Compositarum, in loco Dottabetta, ad Ootacamund, die 19 mensis novembris anni 1955 a N. V. Sundaram, et positus in G. M. Herbario sub numero 2876.

The infection spots are visible on both the surfaces of the leaves as circular yellowish areas with bright orange uredia on the lower surface.

The uredia are entirely covered by the pseudoperidium at the earlier stage, but soon a pore like opening is formed by the rupturing of the central cells of the pseudoperidium.

*Cercospora duddiae* Welles, Welles, Colin B., *Phytopathology* **13**, 362–365, 1923.

A number of oval or elongated spots with slightly raised margin and ash coloured centre develop on the leaves. Smoky brown growth of the fungus is visible on the spots. Dark brown stromata are formed substomatally. The conidiophores are multiseptate, unbranched, light brown, geniculate and are very long measuring up to 180μ long and 9μ broad. The conidia are long tapering at the tip, hyaline, 2 to 15 septate, the septum being formed at short intervals and measure 80 – 162 x 5 – 7μ.

On living leaves of *Allium sativum* (Liliaceae), Kallar, 23–9–55, N. V. Sundaram, G. M. Herb. No. 2867.

This is the first record of this fungus for India.

*Colletotrichum brachytrichum* Delacr. Delacroix, G., *Bull. Soc. Myco. Fr.* **21**, 193, 1905.

Saccardo, P. A. *Syll. Fung.* **21**, 790, 1912.

Many circular to irregular spots starting either from the margin or from the centre of the leaves are formed. When the infection reaches the petiole the leaf gets detached. The centre of the spot on the upper surface is ash coloured with dark brown margin while the lower surface is chocolate brown. The acervuli are formed mostly on the upper surface and are visible as dark coloured erumpent bodies and measure 62 to 186μ broad and 37 to 62μ high. The conidia are oval to ellipsoid, hyaline, one celled and measure 12–19 x 4.5 – 6 μ. Many dark brown, septate, pointed setae measuring 31 – 78 x 4.5 – 6μ are present. The measurements and other characters are in close agreement with the fungus *C. brachytrichum* and hence indentified as such. This is the first record of this fungus for India.

On living leaves of *Theobroma cacao* L. (Sterculiaceae). Kallar Fruit Garden (Coimbatore). 12–11–55, N. V. Sundaram, G. M. Herb. No. 2871.

Arx (1957), however, has made this fungus a synonym of *C. gloeosporioides* Penz.

*Septoria crotalariae* Sund. spec. nov. Spots amphigenous, prominently seen on the upper surface, dark brown, circular with raised margin, 1 to 5 mm. in diam.; pycnidia mostly on the upper surface, minute, spherical with narrow ostiole, 60 to 95μ in diam.; pycnidiae wall made up of 2 to 3 layers of hyaline to sub-hyaline cells; pycnidiospores filiform, hayline, 2 to 4 septate with slightly broader base, 31 x 3.3μ (25–45 x 1.5 – 4.5).

On living leaflets of *Crotalaria madurensis* Wight (Papilionatae), Coonoor, 25–11–56, N. V. Sundaram, G. M. Herb. No. 2865 (Type).

Maculae amphigenae, eminentes in superiore pagina, fusce brunneae, circulares, marginibus elevatis, 1–5 mm. diam.; pycnidia ut plurimum in superiore pagina, minuta, spherica, ostiolo angusto, 60 – 95μ diam.; parietes pycnidiales constantes 2 – 3 seriebus cellularum hyalinarum vel sub-hyalinarum; pycnosporae filiformes, hyalinae, 2 – 4 septatae, ad basin paulo latiores, 31 x 3.3 μ (25 – 45 x 1.5 – 4.5).

Typus lectus in foliolis *Crotalariae madurensis* Wight., e familia Papilionacearum, ad Coonoor, die 25 mensis novembris anni 1956, a N. V. Sundaram, et positus in G. M. Herbrio sub numero 2865.

The spots appear small, irregular and water soaked in its initial formation but become more or less circular and turn dark brown. Due to severe infection, defoliotion occurs.

*Septoria cycadis* Sund. spec. nov. Spots amphigenous, irregular with light brown centre surrounded by chocolate brown discoloration, 2 – 8 mm. in diam.; pycnidia hypophyllous, seen as black dot like bodies with creamy white tendril like growth of the spores, deep seated, spherical or flask shaped, wall made up of 2 – 3 layers of light brown cells, 75 – 93 x 61 – 90μ; conidiophores short, thin, hyaline; conidia hyaline, filiform, 2 – 3 septate, 16 x 3μ (12 – 22 x 2 – 4).

On living leaves of *Cycas circinalis* Linn. (Cycadaceae), Kozhikode (Malabar), 29–8–56, N. V. Sundaram, G. M. Herb. No. 2864 (Type).

Maculae amphigeneae, irregulares, medio pallide brunneo, circumdate discoloratione castaneo-brunnea, 2 – 8 mm. diam.; pycnidia hypophylla, ut corpuscula nigra punctis similia, incremento cremeo-albido circinni simili sporarum, alte infixa, spherica vel ureceolata, parietibus duplici vel triplici serie cellularum pallide brunnearum, 75 – 93 x 61 – 90μ; conidiophorae breves, tenues, hyalinae; conidia hyalina, filiformia, 2 – 3 septata, 16 x 3 μ (12 – 22 x 2 – 4).

Typus lectus in foliis viventibus *Cycadis circinalis* Linn. e familia Cycadacearum, ad Kozhikode, in regione Malabarica, die 29–8–56 a N. V. Sundaram et positus in G. M. Herbario sub numero 2864.

The spots though visible on both the surfaces of the leaves are prominently seen only on the upper surface. The conidia are seen extruded through the ostiole forming tendril like growth especially during the humid weather.

*Septoria zingiberis* Sund. spec. nov. Spots amphigenous, spindle shaped with whitish centre and tawny coloured margin, 2 – 5 mm. broad, many spots coalesce involving major part of the lamina; pycnidia amphigenous, distributed over the papery central portion of the spot, spherical, wall brown coloured, 42 – 123 x 42 – 138μ; conidia borne on short stalks, cylindrical, apex tapering, light brown, 1 – 3 septate, straight or slightly curved, smooth surfaced, 25 x 3μ (12 – 28 x 1.5 – 4).

On living leaves of *Zingiber officinale* Rosc. (Zingiberaceae), Wynaad, 25–9–55, N. V. Sundaram, G. M. Herb. No. 2862 (Type).

Maculae amphigeneae, fusiformes, medio albido, marginibus aurantiacis, 2 – 5 mm. longae, 0.5 – 2mm. latae, earum plures coalescentes atque maximum partem paginae involventes; pycnidia amphigena, distributa per partem papyraceam macularum, spherica, parietibus brunneis, 42 – 123 x 42 – 138$\mu$; conidia singula insidentia brevi stipiti, cylindrica, fastigata ad apicem, pallide brunnea, 1 –3 septata. recta vel paulum curvata, levia, 25 x 3$\mu$ (12 – 28 x 1.5 – 4).

Typus lectus infoliis viventibus *Zingiberis officinalis* Rosc. e familia Zingiberacearum, ad Wynaad, die 25–9–1955, a N. V. Sundaram et positus in G. M. Herbario sub numero 2862.

Due to the severe infection the leaves become blasted and finally dry up. Both young and old leaves are susceptible to infection but the fungus spreads quickly on the young leaves causing them to dry up soon. The pycnidia are found immersed in the dried up papery tissues of the host.

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# PRODUCTION OF MONSTROUS APOTHECIA OF SCLEROTINIA SCLEROTIORUM (LIB.) DE BARY UNDER THE INFLUENCE OF URANIUM NITRATE

Kishan Singh Bedi

(Accepted for publication September, 15, 1961)

Introduction: Worsdell (1915) has discussed monstrous or teratological forms of various fungi and has grouped them in classes, three of which are dichotomy, fasciation and proliferation. He states that botanists believe that the abnormal structures are due to one of the following: pure accident, direct action of environment and phylogenetic or hereditary causation.

Godfrey (1923), working on the gray mould of castor bean, caused by *Sclerotinia ricini,* also observed abnormalities during the development of apothecia. He noticed that the abnormal apothecia were sterile, and attributed these deviations to the intervention of unfavourable conditions of the environment.

Davis (1925) also noticed teratological forms of ascocarps of *Sclerotinia sclerotiorum*.

In his studies on the factors affecting the apothecial development *S. sclerotiorum*, the author observed monstrosities resulting from serious malformations of apothecia and their stipes developing from sclerotia produced under the influence of uranium nitrate in the culture medium.

This paper deals with an acccunt of these monstrositis.

Experimental Results and Discussion: Sclerotia were produced at the laboratory temperature (22° – 25°C.) on potato-dextrose agar containing 0.5, 1.0, 1.5 & 2.0 grams of uranium nitrate per litre. Sclerotia formed on this medium without uranium nitrate served as the control.

For the production of apothecia according to the method developed by Bedi (1956), sclerotia, after they had been air-dried for a couple of days at the laboratory temperature (22° – 25°C), were floated on distilled sterilised water in Erlenmeyer flasks, and incubated at the temperature-range of 15°–20°C. Final observations on the development of apothecia, as recorded at the end of six weeks, are presented in table I.

TABLE 1. Effecte of different concentrations of uranium nitrate in potato-dextrose agar, used to produce sclerotia, on the development of apothecia therefrom.

| Concentration of uranium nitrate per litre of of potato-dextrose agar | Percentage* of sclerotia with stipes only (A) | Percentage* of sclerotia with normal apothecia (B) | Percentage* of sclerotia with abnormal apothecia (C) | Total* percentage germination of sclerotia (A)+(B)+(C) |
|---|---|---|---|---|
| 0 (control ... | 0 | 100 | 0 | 100 |
| 0.5 gram ... | 6 | 88 | 6 | 100 |
| 1.0 gram ... | 10 | 72 | 16 | 98 |
| 1.5 gram ... | 8 | 10 | 82 | 100 |
| 2.0 gram ... | 8 | 0 | 92 | 100 |

*Average of 3 replicates

An examination of the data shows that though the different concentrations of uranium nitrate in the culture medium employed to produce sclerotia do not exercise any influence on the total percentage germination, yet they are productive of profound effects on the stipes and apothecia developing therefrom (Fig. 1). It may be noticed that as the concentration of this

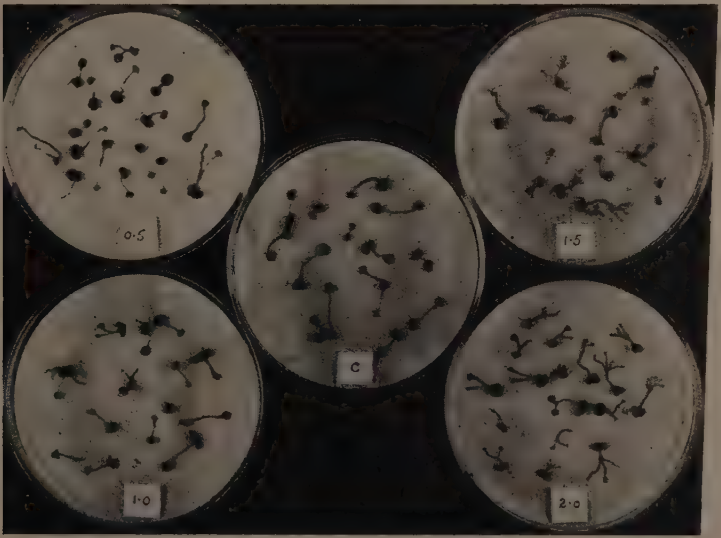


Fig. 1. Effect of different concentrations of uranium nitrate in potato-dextrose agar, used to produce sclerotia, on the development of apothecia therefrom. (C=Control)

radioactive substance in the medium rises, the number of normal apothecia decreases progressively and in place of them there are produced monstrosities of various shapes and kinds. It may be pointed out that no asci and ascospores were produced in any of the abnormal or freakish structures produced, thus corroborating the finding of Godfrey (1923) in the case of *S. ricini*. Whereas the control sclerotia produced on potato-dextrcse agar without uranium nitrate are producing normal apothecia, one at the top of each stipe. In all concentrations of uranium nitrate, usually each stipe bears two apothecia, thus exhibiting the phenomenon of dichotomy. At the highest concentration of two grams of this chemical per litre of the medium, all the apothecia are abnormal. At this concentration, the stipe from one sclerotium has at its swollen tip 7 small, abortive or rudimentary apothecia in a cluster with secondary stipes.

## Summary

Though the different concentrations of uranium nitrate in the culture medium employed to produce sclerotia do not affect their total percentage germination, yet profound effects are produced in the morphology of stipes and apothecia emerging therefrom. As the concentration of this radioactive substance increases in the culture medium, the number of normal apothecia decreases progressively and there are formed instead only monstrosities or freakish structurs of various shapes and kinds. Such malformed or abnormal structures remain sterile, producing no asci with ascospores.

Acknowledgement: The author is very much indebted to Dr. E. C. Stakman, Professor Emeritus, under whose stimulating and masterly guidance the investigation was pursued at the University of Minnesta, St. Paul, U.S.A.

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## Phytopathological Note

**Efficacy of different fungicides IV. Field trials for the control of grain smut of jowar (Sphacelotheca sorghi (Link) clinton):—** J. S. Grewal and Dharam Vir: In comparative field trials carried out for 3 years for the control of grain smut of *jowar*, seeds of variety Kanpur white, artificially inoculated with chlamydospores of *Sphacelotheca sorghi* at the rate of 0.5 per cent of seed weight, were treated with nine different fungicides i.e. Arasan (tetramethylthiuram disulphide), N. 1. Ceresan (ethylmercury phosphate), Tillex (ethylmercury chloride), Flit 406 n–(trichloromethylthio)–4–cyclohexene–1, 2–dicarboximide, Fusariol (ethylmercury cyanide), Fernasan A (tetramethylthiuram disulphide), Agrosan GN (tolylmercury acetate), Sulphur and Ceresan M (ethylmercury p-toluene sulfonanilide) at the rate of 0.25, 0.12, 0.20, 0.25, 0.25, 0.22, 0.30, 0.50 0.22 per cent of seed weight respectively. All treatments were replicated four times and counted number of seeds were sown in all plots in order to see the effect of fungicides on germination of seed. It was found that all fungicides improved germination as compared with control, and Ceresan M gave best results.

The disease incidence was recorded on the basis of smutted heads. There was no disease in the plots sown with fungicide-treated seeds while in the untreated ones the incidence of smut was 6.9, 24.5 and 21.2 percent respectively for the three consecutive years. The results further indicate that *Sphacelotheca sorghi* is a very sensitive fungus and is controlled by all the mercurial and non-mercurial fungicides tried for its control in the present trials.

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Contributions should be on one side of the page, double spaced, with a 1¾th inch margin on the left. In form and style, such as punctuation, spelling and use of italics, the manuscript should conform to the best Journals in the U.K. and U.S.A. Authors should strive for a clear and concise style of writing. The name and address of the Institution at which the work was done should be cited immediately after the SUMMARY at the end of the article on left hand side. Tables should be numbered and each table should have a heading stating briefly its contents. References to literature should be made as foot notes *only* when four or fewer citations are given. If there are more, they should be listed under 'REFERENCES' at the end of the paper and referred to by date in brackets in the body of the article. Citation should give the name of the author (or authors), his (or their) initials, year of publication and then the full title correctly, followed by the name of the Journals, number of the volume, a colon and page numbers. If the title is in a foreign language, then diacretic signs and capitalization should be precisely as in the original. The names of the Journal should be as abbreviated in the WORLD LIST OF PERIODICALS, 2nd Ed., 1934, but as the book may not be available to all, contributors are requested to give the titles in full. Abbreviating will in that case, be done by the Editors. If an article has not been seen in original, then that fact should be clearly stated. An example citing is given below:—

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Authors are invited to consult Bisby's 'An Introduction to Taxonomy and Nomenclature of Fungi' (1945), p.p. 38-41 and Riker's "The preparation of manuscripts for Phytopathology, *Phytopathology* 36 : 953-977, 1946, before preparing their mss. and figures.

Articles will be published in the order of their approval for publication but the address of the retiring President and invitation articles will be published when received.

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# INDIAN PHYTOPATHOLOGICAL SOCIETY



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